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# Monoclonal Antibody Production Via Fluidized Bioreactor Technology

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# Monoclonal Antibody Production Via Fluidized Bioreactor Technology

## **Abstract**

Monoclonal antibodies (mAbs) are biologically identical antibodies created by homogenous immune cells originating from the same parent cell. MAb target a specific epitope of an antigen on a cell's surface, allowing it to neutralize the antigen. This unique characteristic has made them a key tool in the biopharmaceutical industry for the production of therapeutic drugs. One of these drugs is Rituxan® (rituximab), a mAb drug for the treatment of various cancers and autoimmune diseases. Currently, most mAb products are grown via cell suspension technology in stirred tank bioreactors. However, we have found that by using an integrated bioprocessing model, including conventional cell suspension culture tanks and fluidized bioreactor technology, overall product yield per day is increased by about 7-fold for the production of Rituxan®. Additionally, an economic analysis shows the fluidized bioreactor process is more profitable. Furthermore, though it requires a higher initial investment than the stirred tank process, the differential present worth of the fluidized bioreactor process in comparison to the stirred tank process is \$13 billion. Overall, for the production of Rituxan®, the use of fluidized bioreactor technology is a more productive and lucrative process than the conventional stirred tank process.

## **Disciplines**

Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering

# **MONOCLONAL ANTIBODY PRODUCTION VIA FLUIDIZED BIOREACTOR TECHNOLOGY**

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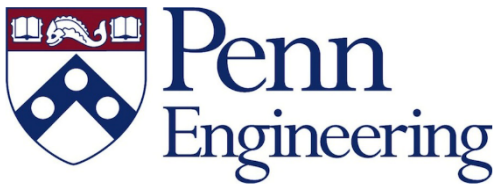
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April 23, 2014







April 23, 2014

Professor Leonard Fabiano  
University of Pennsylvania  
School of Engineering and Applied Science  
Department of Chemical and Biomolecular Engineering  
220 S. 33<sup>rd</sup> Street  
Philadelphia, PA 19104

Dear Professor Fabiano,

Enclosed herewith is a detailed process design and economic analysis comparing two methods of monoclonal antibody production. The traditional stirred tank bioreactor process currently used in industry was compared to a hybrid process involving a fluidized bioreactor. A fluidized bioreactor process was chosen as comparison because it allows for greater product formation and ultimately a more profitable method to produce generic rituximab. The production goal was set at 650kg of generic Rituxan<sup>®</sup> (rituximab), a monoclonal antibody currently sold by Roche. However, it was determined that five parallel processes of the stirred tank process would be required to meet the production requirement while only one fluidized bioreactor process is necessary.

The report provides significant details regarding the process unit operations along with a detailed design of the fluidized bioreactor, which is a new, unique and difficult concept. Furthermore, a fluidized bioreactor model was also developed to model the growth of cells in the environment along with the effect of various factors on product formation.

Based on the economic analysis, we recommend implanting the fluidized bioreactor process to create product at the lowest dollar and time cost. This process can produce 650 kg of product (after downstream purification) in 212 days. This design process will require an initial capital investment of about \$1.8 billion. The project will have an internal rate of return (IRR) of 225% and a net present value (NPV) of \$17 billion, assuming a 10-year operational life.

All calculations for the report were done based on literature values. Please contact us regarding any questions or concerns that you may have about either process covered in the report.

Best,

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Lydia Atangcho

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Meghan McCullough

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Shenali Parikh

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Alexandra Stambaugh



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## 1. Abstract

Monoclonal antibodies (mAbs) are biologically identical antibodies created by homogenous immune cells originating from the same parent cell. MAbs target a specific epitope of an antigen on a cell's surface, allowing it to neutralize the antigen. This unique characteristic has made them a key tool in the biopharmaceutical industry for the production of therapeutic drugs. One of these drugs is Rituxan<sup>®</sup> (rituximab), a mAb drug for the treatment of various cancers and autoimmune diseases. Currently, most mAb products are grown via cell suspension technology in stirred tank bioreactors. However, we have found that by using an integrated bioprocessing model, including conventional cell suspension culture tanks and fluidized bioreactor technology, overall product yield per day is increased by about 7-fold for the production of Rituxan<sup>®</sup>. Additionally, an economic analysis shows the fluidized bioreactor process is more profitable. Furthermore, though it requires a higher initial investment than the stirred tank process, the differential present worth of the fluidized bioreactor process in comparison to the stirred tank process is \$13 billion. Overall, for the production of Rituxan<sup>®</sup>, the use of fluidized bioreactor technology is a more productive and lucrative process than the conventional stirred tank process.

## 2. Introduction

### 2.1. Project Background

#### 2.1.1. Rituxan<sup>®</sup>

While most mAbs are made on a small to medium scale for laboratory use, successful mAb drugs used for medicinal treatment are responsible for a multibillion-dollar industry with each mAb responsible for at least \$1 million in sales for its respective companies (U.S. Pharmaceutical Sales, National Research Council). For the larger blockbuster drugs, the sales of mAb may bring in billions every year. In such large domestic and international markets, there is great potential for generics. For the purpose of this project, we will examine the production optimization for Rituxan<sup>®</sup> (rituximab). By developing a more profitable process, a generic drug can be produced at a lower cost.

Rituxan<sup>®</sup> (rituximab) is a CD-20 monoclonal antibody. It has an affinity for cells that overexpress the CD-20 target receptor, a common sign of non-Hodgkins lymphoma, Crohn's disease, chronic lymphocytic leukemia, and rheumatoid arthritis. Rituxan<sup>®</sup> depletes the body's B-cells. It has three proposed mechanisms of action: complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity and apoptosis. Though the mechanisms are not fully understood, all three essentially require the mAb to bind to immune system B-cells by recognizing the protein receptor, CD-20, after which the drug can successfully kill the target cell (Proposed Mechanism of Action).

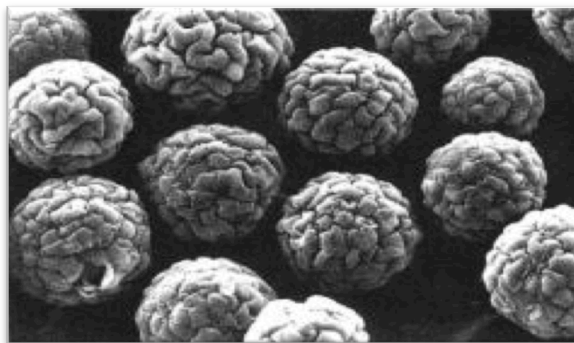
### **2.1.2. Fluidized Bioreactor Process**

Since the introduction of mAbs to the field of therapeutic medicine, factories have adapted their designs to produce pure, high-quality mAbs. However, because mAbs require strict purification processes and a moderately long production process in comparison to small molecule drugs, plants are generally over-capacity. MAb production is dependent significantly on the cell culture conditions, which commonly include growing cells in suspension. Suspension culture is widely used because it presents the simplest way of achieving a high cell density for a maximum product yield. With larger batches, larger-scale purification is necessary, but cannot be accommodated by current plant designs (Kelley 450). We propose to introduce a different cell culture technique for application in large-scale mAb production – one that will provide several useful advantages over traditional suspension culture techniques.

Many mammalian cell lines, including those most commonly used for mAb production, such as Chinese Hamster Ovary (CHO) cells, are anchorage dependent unless modified for suspension culture growth. By utilizing microcarriers, small porous particles capable of remaining in suspension, cells can grow in psuedo-suspension by adhering on or inside these particles. Two major advantages of this are increased production capacity and reduced labor requirement in comparison to traditional suspension culture processes. Due to a high surface area to volume ratio, the use of microcarriers provides higher cell and product yields than is possible with a traditional suspension bioreactor. Another advantage of using microcarriers is easier downstream purification. Because cells are retained on the carriers and product is secreted into the medium, separation of cells and product is achieved by a simple filtration step instead of the typical centrifugation step, a

more costly process. Furthermore, microcarriers help protect against physical and chemical stress. Since the cells grow inside the porous beads, they are protected from shear stresses caused by the tip of the stirrer as well as the sparged oxygen bubbles. Additionally, cells tolerate more chemical stress such as lactate build up when growing inside the porous carriers (Rodrigues, et al.) (GE Microcarrier Handbook).

In the proposed design, a fluidized bioreactor with GE Cytoline™ 1 microcarriers is used as the main production step for the product. These beads, shown in Figure 2.1, are ellipsoid particles with 0.4-1.1mm thickness, 1.7-2.5mm length, and 10-400µm pores. A recombinant CHO cell line will be used as the mAb-producing cell line. Though specific cell productivity is likely to be less than that of the cell suspension process, overall productivity will be higher due to a higher sustainable cell density. A traditional stirred tank process that utilizes stirred tank bioreactors for both seed train and production steps to a new process containing a near-identical seed train and a fluidized bioreactor as the production step.

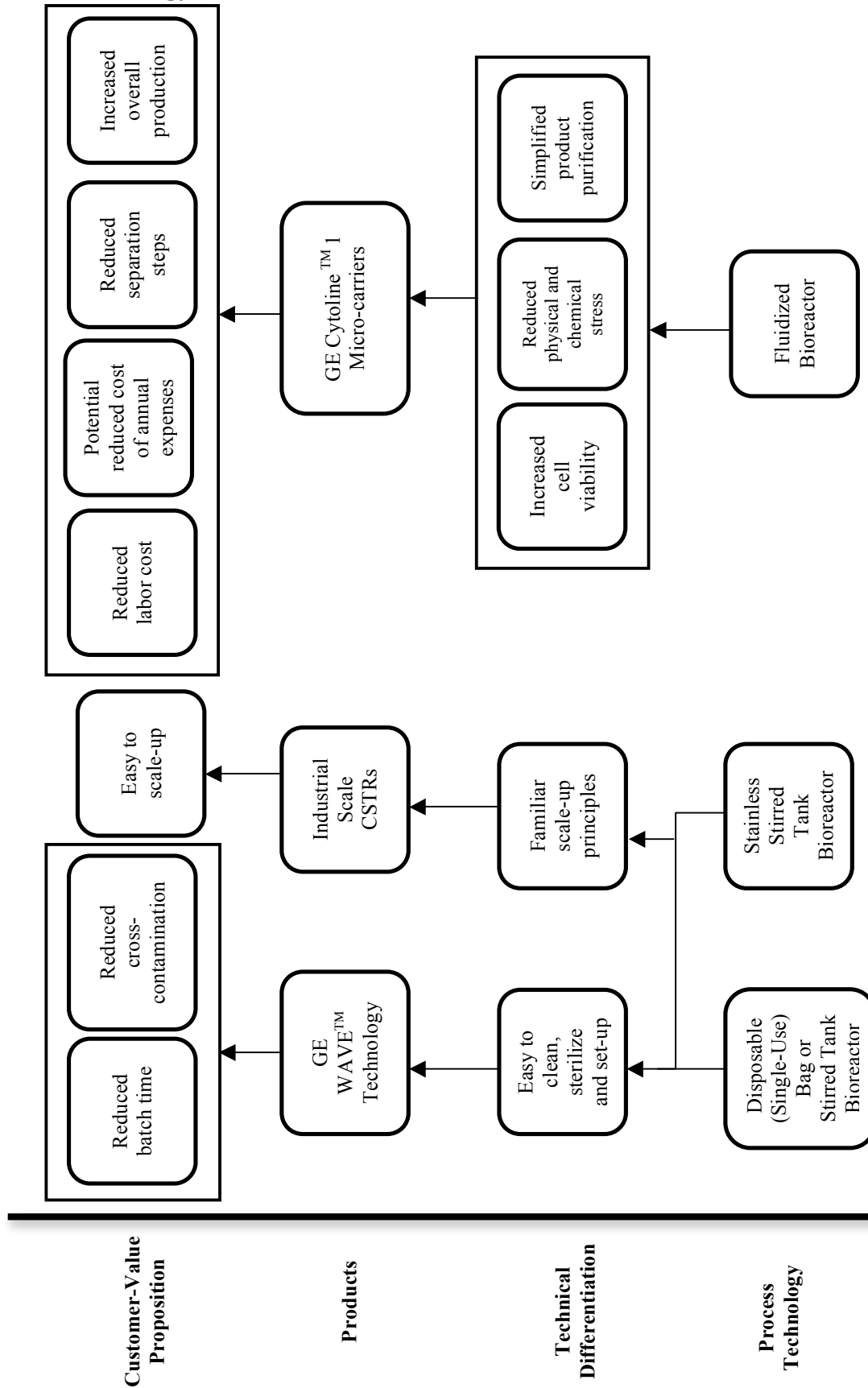


**Figure 2.1 Cytoline 1 Microcarriers Covered with Cells**

## 2.2. Project Charter

<b>Project Name</b>	Monoclonal Antibody Production via Fluidized Bioreactor Technology
<b>Project Leaders</b>	Lydia Atangcho, Meghan McCullough, Shenali Parikh, Alexandra Stambaugh
<b>Specific Goal</b>	Design a biopharmaceutical plant and process to produce a generic version of Rituxan <sup>®</sup> (rituximab) using a fluidized bioreactor as the final production step rather than using a traditional cell suspension culture.
<b>Project Scope</b>	<p><u>In Scope:</u></p> <ul style="list-style-type: none"> <li>• Manufacturing process for rituximab beginning from prepared inoculum of cells to final growth step               <ul style="list-style-type: none"> <li>○ Design main process to include fluidized bioreactor technology as the final production step                   <ul style="list-style-type: none"> <li>▪ Expected increase in overall volume productivity</li> </ul> </li> <li>○ Design a traditional industry standard process without fluidized bioreactor steps as a basis for comparison</li> </ul> </li> <li>• Post-manufacturing purification steps beginning from the first harvest step (centrifugation or filtration) to final sterile filtration</li> <li>• Meet current safety and health regulations for biological products</li> <li>• Maintain process integrity and compliance by adhering to good manufacturing practices (GMP)</li> <li>• Determine which process (fluidized v. traditional) is best when compared on basis of amount of drug produced per time per dollar</li> </ul> <p><u>Out of Scope:</u></p> <ul style="list-style-type: none"> <li>• Research and development of generic rituximab (produced in laboratory)</li> <li>• Cell line development</li> <li>• Clinical trials</li> <li>• Packaging and distribution of drugs</li> <li>• FDA approval of generic drug</li> <li>• FDA approval of use of large-scale fluidized</li> </ul>
<b>Deliverables</b>	<p>Business opportunity assessment</p> <ul style="list-style-type: none"> <li>• What is the market for generic production of Rituxan<sup>®</sup> and monoclonal antibodies in general?</li> <li>• How does the fluidized bioreactor process compare to the traditional process?</li> </ul> <p>Technical feasibility assessment</p> <ul style="list-style-type: none"> <li>• Is it technically feasible to manufacture antibodies on a large scale using a fluidized bioreactor setup?</li> </ul> <p>Manufacturing capability assessment</p> <ul style="list-style-type: none"> <li>• Can this facility be built and the process utilized without significant capital investment?</li> <li>• Will the process satisfy the stringent FDA requirements for a pure, consistent product?</li> </ul>
<b>Time Line</b>	Facility and process design along with economic analysis within four months.

## 2.3. Technology Readiness Assessment



### 3. Concept Stage

In 2013, the sales of Rituxan<sup>®</sup> reached over \$3.2 billion in the US market and \$2.12 billion in the European market. Since its introduction in the US market in 2011, Rituxan<sup>®</sup> has experienced a general upward trend in sales, which is expected to continue until its patent expiration in 2014 in Europe and in 2018 in the United States. In general, the mAb market is expected to have a compound annual growth rate of about 5.3% indicating almost \$58 billion in sales globally (U.S. Pharmaceutical Sales).

With an increase in monoclonal antibody drugs in the pipelines of big pharmaceuticals such as Genentech and Abbott Labs<sup>™</sup> and in increasing global demand for the drugs, current production facilities must be able to meet the necessary production goals. As previously mentioned, current facilities often struggle to produce more than a certain amount of antibody per batch due to the restrictions of maximum cell density in a cell suspension culture. When a fluidized bioreactor is used as the final upstream step, the cells can adhere to the microcarriers and grow to a 100 times higher sustainable cell density than the traditional process. This allows for an overall increase in cell productivity.

#### 3.1. Competition in the Generic Market and Production Goal

Before drugs lose their patent protection, generic manufacturers have already begun to manufacture a competitor for the market, which is ready to launch as soon as the patent expires. Currently, Roche expects to maintain exclusivity in the international market well into 2015 because no other company has been able to gain approval for generic Rituxan<sup>®</sup>. This opens up a great opportunity to potentially build a more lucrative and more productive process and gain hold of the rituximab market once Roche's patent expires. However, Teva Laboratories may become a competitor since their mAb is in Phase III clinical trials (Roche: Impact Of Rituxan Patent Expiration In Europe).

In 2013, over 2.2 million units of Rituxan<sup>®</sup> were sold in the US alone amounting to \$3.2 billion in US revenue. In Europe, Rituxan<sup>®</sup> earned about \$2.1 billion in revenue. Based on the units of Rituxan<sup>®</sup> sold (each unit refers to either a 100mg/10mL or 500mg/50mL vials) and the average price per dose of \$586 (100mg/10mL vial) and \$2,840 (500mg/50mL vial), a production goal can be determined. Assuming that the facility will eventually produce enough product to capture 70% of the domestic and European markets, 650 kg of generic

rituximab will ultimately become the production goal (see Section 13.1) (U.S. Pharmaceutical Sales).

### **3.2. Process and Facility Requirements**

The new facility will be built on a brownfield site on the Delaware River. The East Coast is the preferred location for the facility because it allows the facility easy access to land and sea transportation. Furthermore, the Eastern seaboard, and in particular, the Philadelphia and Delaware areas have become a hub of innovation for the biotechnology field. The brownfield site will be retrofitted to match the desired needs of the new design. It will also help reduce the initial investments cost for the land and building as well as equipment. Rather than purchasing equipment, an annual fee can be paid in order to utilize the preexisting equipment at the site.

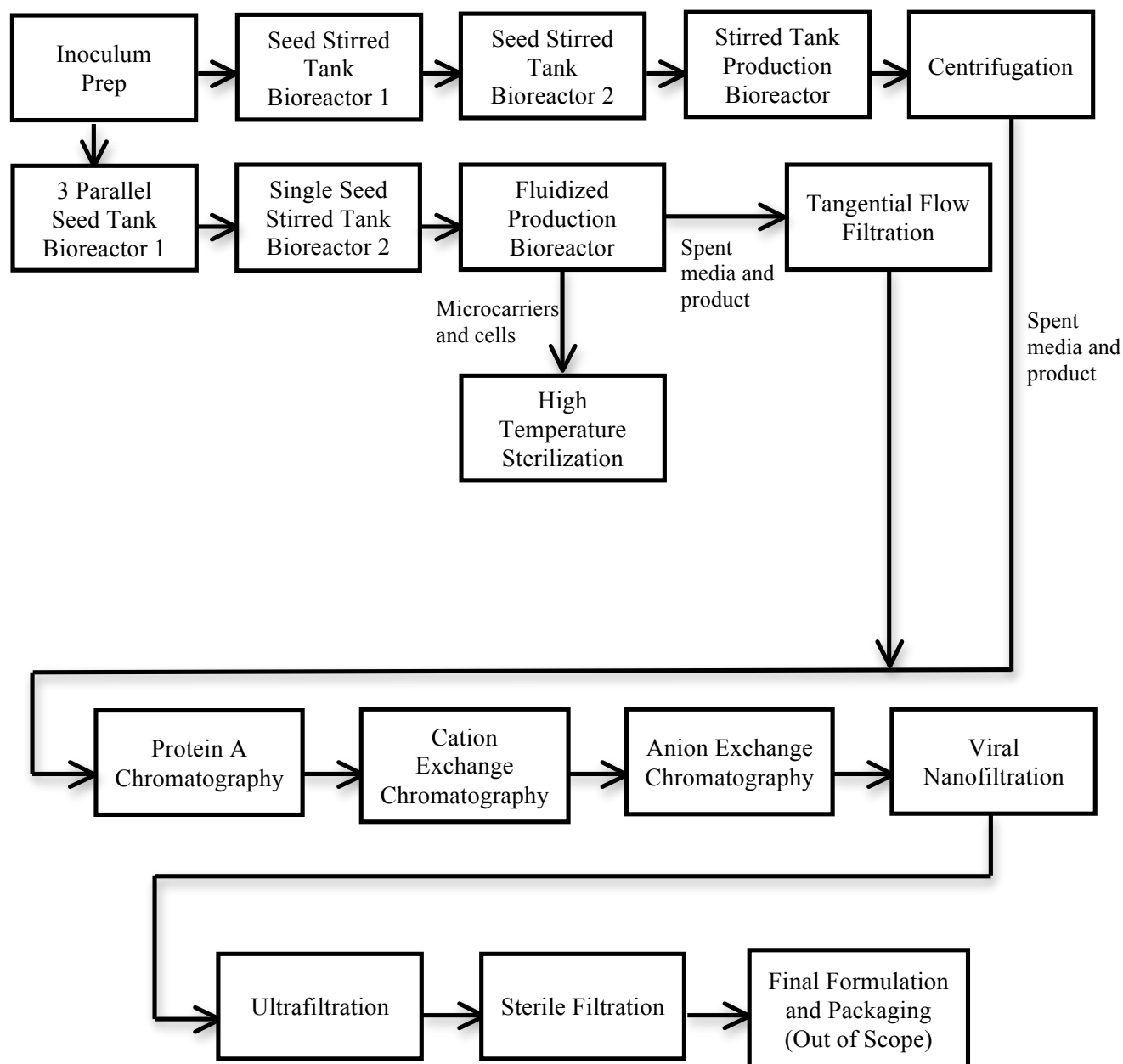
The new facility requires a new-unique-and difficult design concept. The facility is being designed for a company attempting to enter the generic monoclonal antibody market wishing to specifically produce rituximab. Though out of scope for this project, the proposed designs can be used to produce any mAb allowing for some minor changes. The new facility will be equipped with the most updated, cost-effective equipment. Specifically, the facility will be equipped with disposable unit operations wherever applicable. The rising trend of using disposable bioreactors and tanks, the disposable route offers shorter batch times, eliminates SIP and CIP procedures, and provides long-term savings. According to a report of A. Sinclair, single-use bioreactors will help to save 30% of electrical energy for operation, 62% of the energy input for the production of the system, 87% of water and finally 95% of detergents, all compared to conventional bioreactors.<sup>1</sup>

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<sup>1</sup> Sinclair A., Leveen L. et al. (2008). "The Environmental Impact of Disposable Technologies". *BioPharm International Supplements*.

### 3.3. Block Flow Diagram

The diagram below depicts the overall process design for the stirred tank bioreactor and fluidized bioreactor processes. The overall processes are very similar, but the fluidized bioreactor process has a slightly different downstream purification process.

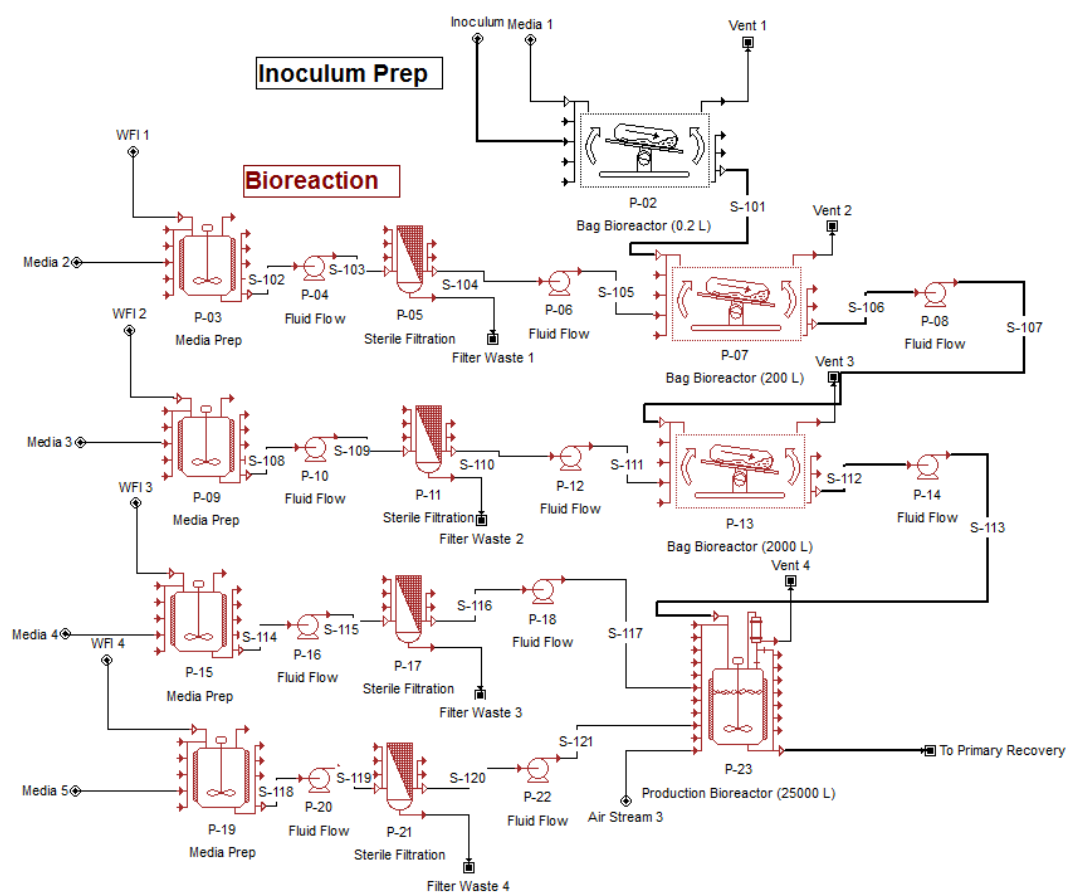




## 4. Process Flow Diagrams

The following pages include the process flow diagrams (PFD) for the two main processes: monoclonal antibody production via traditional stirred tank bioreactor process and production via the fluidized bioreactor process. The overall diagram for each process is divided into two sections: production and purification of product.

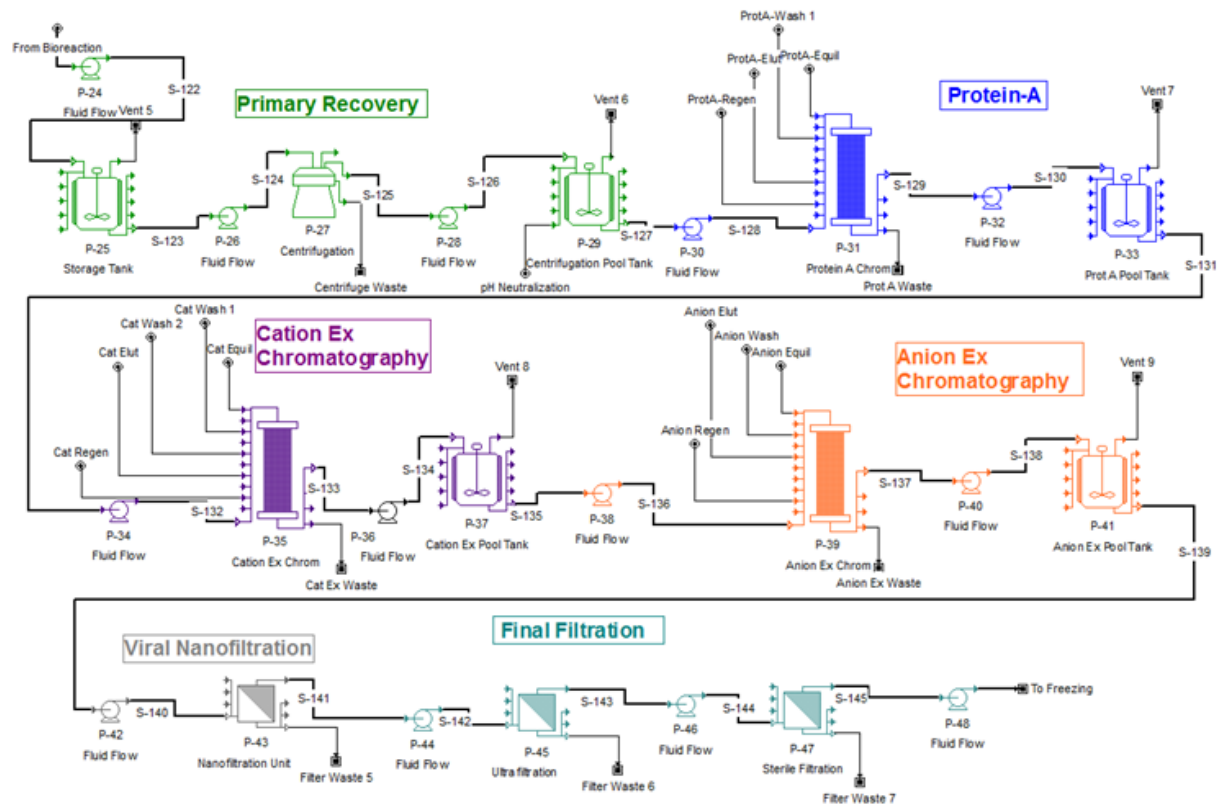
### 4.1. Production PFD for Stirred Tank Bioreactor Process (PFD 01)



#### 4.1.1. Overall Mass Balance for PFD 01

PFD 01- Stirred Tank Inoculum Prep & Bioreaction		
Component	Input (kg/batch)	Output (kg/batch)
Cells	0.00524	356.32
Media	0.002	0
Water	0.098	19600
Endotoxin	0	36.34
Product	0	9.41

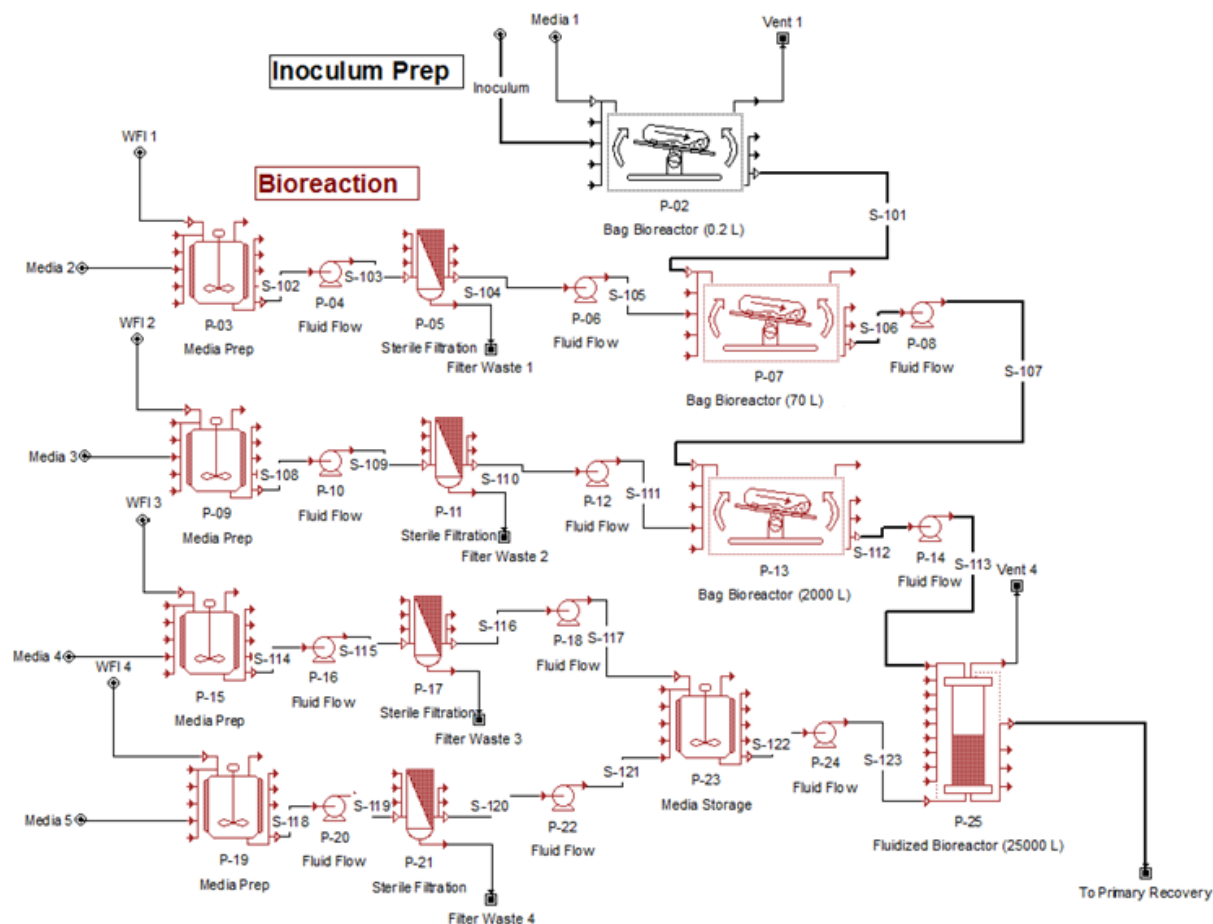
4.2. Purification PFD for Stirred Tank Bioreactor Process (PFD 02)



4.2.1. Overall Mass Balance for PFD 02

PFD 02- Stirred Tank Downstream Purification		
Component	Input (kg/batch)	Output (kg/batch)
Cells	356.32	0.0
Media	0.0	0.0
Water	19600	2000
Endotoxin	36.34	0.0
Product	9.41	7.76

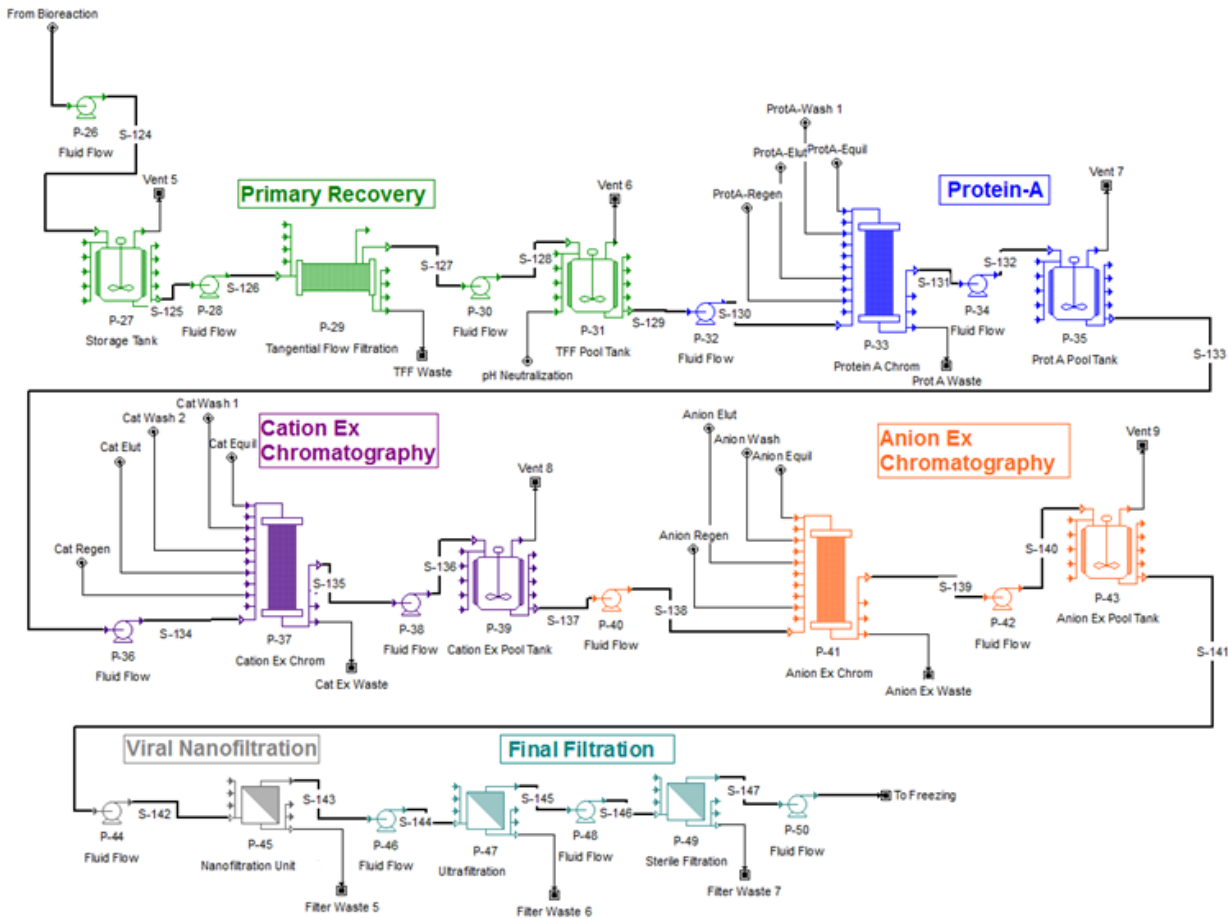
### 4.3. Production PFD for Fluidized Bioreactor Process (PFD 03)



#### 4.3.1. Overall Mass Balance for PFD 03

PFD 03- Fluidized Bioreactor Inoculum Prep & Bioreaction		
Component	Input (kg/batch)	Output (kg/batch)
Cells	0.00524	2672.4
Media	0.002	$1.17 \times 10^5$
Water	0.098	$5.90 \times 10^6$
Endotoxin	0.0	272.58
Product	0.0	65.75

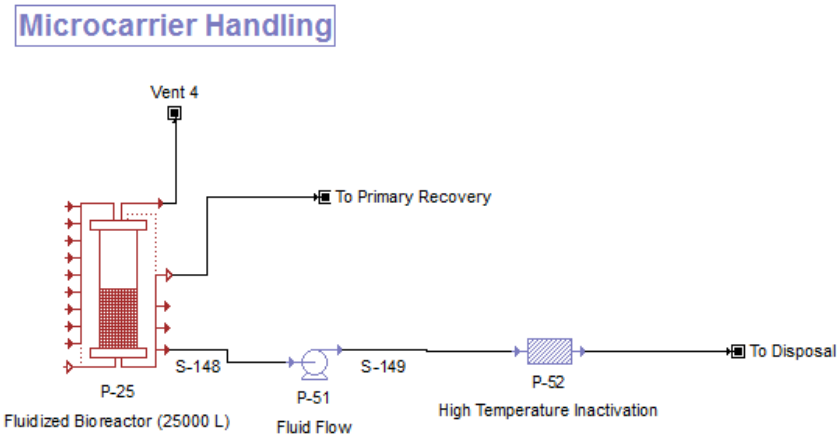
4.4. Purification PFD for Fluidized Bioreactor Process (PFD 04)



4.4.1. Overall Mass Balance for PFD-04

PFD 4- Fluidized Bioreactor Downstream Purification		
Component	Input (kg/batch)	Output (kg/batch)
Cells	2672.4	0.0
Media	$1.17 \times 10^5$	0.0
Water	$5.90 \times 10^6$	2000
Endotoxin	272.58	0.0
Product	65.74	54.21

4.5. Microcarrier Handling PFD for Fluidized Bioreactor Process (PFD 05)



## 5. Production Process Description

All seeding densities for cells were calculated to minimize cell growth time while maximizing production rate (See Appendix F).

### 5.1. Media Preparation – Stirred Tank and Fluidized Bioreactor Process

Both processes will use Ex-Cell<sup>®</sup> ACF CHO Media from Sigma-Aldrich<sup>™</sup>. This is chemically defined, serum-free, and protein-free media specially formulated for optimal CHO cell growth. For efficiency, prepared media will be purchased directly from Sigma-Aldrich<sup>™</sup> for the inoculum preparation steps. For the bioreactors the media will be prepared in various mixing tanks (refer to process flow diagrams) by adding dry powder media to water-for-injection (WFI). The prepared media solution will be filtered with a 0.2 micron sterile filter to remove any foreign particles and large viruses. The media concentration will remain at about 20 grams of dry media per liter of prepared solution.

### 5.2. Cytoline<sup>™</sup> Microcarrier Prep – Fluidized Bioreactor Process Only

To prepare the Cytoline<sup>™</sup> 1 beads for sterilization, two volumes of distilled water are added to one volume of microcarrier beads. Then, the mixture is degassed and autoclaved for 10 minutes at 121°C (1 bar). The water supernatant is removed with a sterilized pipette. Fresh distilled water is added and the microcarriers are stirred for 10 minutes. Again, the water supernatant is removed and 0.1M NaOH is added. The mixture is then incubated overnight and washed with distilled water until the pH stabilizes. Next, the microcarriers are autoclaved in distilled water or PBS at 121°C (1 bar) for 30 min.

### 5.3. Inoculum Preparation Section

#### 5.3.1. Stirred Tank Bioreactor Process

To prepare the inoculum for seed bioreactor, twenty 0.2L disposable bag bioreactors with 0.1 L working volume will each be inoculated with 2.0mL of cells at a concentration of  $1 \times 10^8$  cells/mL. Fifty percent of the total volume is used to allow for proper agitation of the culture and allow for ample air space. The culture will require about 3.5 days to grow to the desired cell density of  $1.0 \times 10^7$  cells/mL. The temperature is

monitored and controlled at 37°C, the pH at 7.0 and the dissolved oxygen level at 50% via PID (positive-integral-differential) feedback controls.

### **5.3.2. Fluidized Bioreactor Process**

To prepare the inoculum for the seed bioreactor, thirty 0.2L disposable bag bioreactors with 0.1L working volume will each be inoculated with 2.0mL of cells at a concentration of  $1 \times 10^8$  cells/mL. Fifty percent of the total volume is used to allow for proper agitation of the culture and allow for ample air space. The culture will require about 3.5 days to grow to the desired cell density of  $1.0 \times 10^7$  cells/mL. The temperature is monitored and controlled at 37°C, the pH at 7.0 and the dissolved oxygen level at 50% via PID (positive-integral-differential) feedback controls.

## **5.4. Seed Bioreactor Section**

### **5.4.1. Stirred Tank Bioreactor Process**

At the end of the inoculum preparation, 98L of prepared and sterilized media solution will be added to a 200L bag seed bioreactor with 100L working volume. The cell cultures from all inoculum bag bioreactors will be transferred via a peristaltic pump to the seed bioreactor. The culture will be rocked continuously to ensure proper mixing. The culture will be allowed to grow for four days with a target final cell density of  $1.12 \times 10^6$  cells/mL. Assuming a product growth rate of 90 pg/cell/day, about 24 grams of antibody product will be produced. Throughout the process, the temperature will be monitored and controlled at 37°C, the pH at 7.0, and the dissolved oxygen level at 50% via PID controls.

After the culture in the first seed bioreactor is allowed to grow for 4 days, the contents will be transferred via peristaltic pump to the 2,000L second bag seed bioreactor containing 900L of sterilized media solution. The second seed bioreactor will have a working volume of 1,000L. The culture will be rocked continuously to ensure proper mixing. The culture will be allowed to grow for 4 days with a target final cell density of  $6.29 \times 10^5$  cells/ml. Over this time, 134 grams of antibody product will be produced. Throughout the process, the temperature will be monitored and controlled at 37°C, the pH at 7.0, and the dissolved oxygen level at 50% via PID controls.

#### **5.4.2. Fluidized Bioreactor Process**

At the end of the inoculum preparation, the cell cultures from 0.2L bag bioreactors will be distributed equally (via sterile tubing) to three 200L bag seed bioreactor with a working volume of 100L (running in parallel). This design setup was chosen to help limit the cell growth time to 5 days but still provide enough cells to inoculate the second seed bioreactor. Upon transfer, the bag bioreactors will already contain 98L of prepared and sterilized media solution. The culture will be rocked continuously to ensure proper mixing. It will be allowed to grow for four days with a target final cell density of  $2.0 \times 10^6$  cells/mL. Assuming a product growth rate of 90 pg/cell/day, about 51 grams of antibody product will be produced. Throughout the process, the temperature will be maintained at 37°C, the pH at 7.0, and the dissolved oxygen level at 50% via PID controls.

After 5 days, the culture from all three reactors is transferred into a single 2000L seed bioreactor with a 1000L working volume. Upon transfer, the bioreactor will contain 900L of prepared and sterilized media solution will be immediately added to the seed bioreactor. The culture will be rocked continuously to ensure proper mixing. The culture will be allowed to grow for 5.5 days with a target final cell density of  $1.5 \times 10^6$  cells/mL. Over this time, 409 grams of antibody product will be produced. Throughout the process, the temperature will be maintained at 37°C, the pH at 7.0, and the dissolved oxygen level at 50% via PID controls.

### **5.5. Production Bioreactor Section**

While the seed bioreactor section served to grow up the culture, the final bioreactor serves to maximize product formation.

#### **5.5.1. Stirred Tank Bioreactor Process**

For the traditional stirred tank bioreactor process, the cell culture will be transferred via a peristaltic pump into the 25,000L production bioreactor with a working volume of 20,000L. Upon transfer, 19,000L of prepared and sterilized media solution will have been immediately added to the seed bioreactor. The culture will be stirred continuously to ensure proper mixing. The culture will be allowed to grow for 16 days with a target final cell density of  $6.11 \times 10^5$  cells/mL. Assuming a productivity rate of 90



pg/cell/day, 9.3 kg of antibody product will be produced. Throughout the process, the temperature will be maintained at 37°C, the pH at 7.0, and the dissolved oxygen level at 50% via PID controls.

### 5.5.2. Fluidized Bioreactor Process

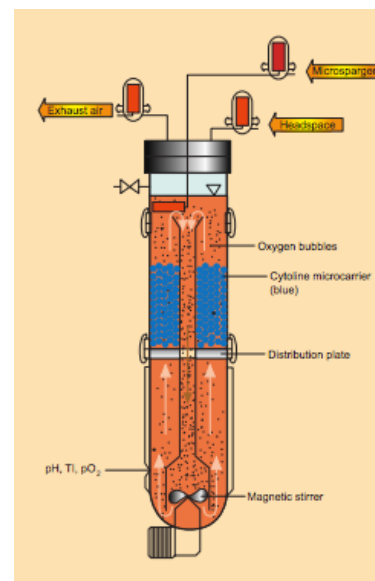
The reactor must be inoculated with the cells from the previous seed bioreactor steps, which will first have to adhere to the Cytoline™ 1 beads. These cells should be in a logarithmic growth phase and in a good nutritional state. This adherence protocol is a continuation upon the microcarrier prep protocol (Section 5.2). The culture medium is first removed completely from the equilibrated microcarrier-cell culture medium mixture. The cells from the previous seed bioreactor along with 9,000L of fresh culture medium are added to 730L Cytoline™ 1 beads in the fluidized bed bioreactor. The culture will have an initial cell density of  $2.0 \times 10^6$  cells/mL microcarrier, as specified by the GE Microcarrier and Cell Culture Handbook. The mixture is agitated gently every 30 minutes for a total of 5 hours. After 5 hours, culture medium is added to the final working volume of 20,000L and incubated overnight while stirred at 100 rpm.

The fluidized bioreactor will then be operated to circulate media while spinning at approximately 300 rpm, which corresponds to a flow rate of about 19 cm/min of media. The culture media will circulate through the bottom of the annular fluidized bed bioreactor. From the top, about 78% of culture media will be removed continuously and moved to a holding tank. The remaining 27% will be circulated back through the internal circulation loop to be enriched in the bottom of the reactor and circulated through again. The culture will be allowed to grow for 12.8 days with a final cell target density of  $1.4 \times 10^8$  cells/mL microcarrier. Assuming a cell productivity rate of 90 pg/cell/day, 65 kg of antibody product will be produced. Throughout the process, temperatures will be maintained at 37°C, pH of 7.0, and the dissolved oxygen level at 40% via PID controls.

## 5.6. Theoretical Model for Fluidized Bioreactor Process

The fluidized bioreactor, shown in Figure 5.1, is a unit that is fluidized with an internal circulation system. This component is modeled as a combination of a Cytopilot™ (Vogelbusch GmbH Vienna) and a typical annular fluidized bed reactor. As with the Cytopilot™, this fluidized bed bioreactor will have two components: an upper cylindrical

chamber and a lower cylindrical chamber. The lower chamber is outfitted with a double-jacket heating circuit, sampling and discharge facilities, a magnetic stirrer, and pH and dissolved oxygen probe nozzles. The media is agitated by the magnetic stirrer and delivered through the distributor plate to the microcarriers in the upper chamber of the fluidized bioreactor. The hydrodynamic pressure lifts the settled microcarriers to form a fluidized bed, and the chamber is tall enough such that there is an apparent separation between the top of the fluidized microcarriers and the top of the media volume. A portion of the media flows down the channel that defines the internal circulation loop, where it will be deposited back into the lower chamber. Micro-bubbles of oxygen are sparged into the downflow of the internal circulation tube and distributed homogeneously by the impeller in the lower chamber. The working volume of the annular fluidized bed in the upper chamber was designed to be 20,000L with an aspect ratio of height to diameter of 4:1. The bed has a diameter of 2 meters and a height of 8.13 meters, which is reasonable for an industrial scale bioreactor.



**Figure 5.1 Fluidized Bioreactor**

### 5.6.1. Fluidization Velocity

Practical operations of the fluidization stream in typical annular fluidized beds are conducted at two or more multiples of the fluidization velocity. The maximum flow rate of the bioreactor is dictated by the sedimentation rates of the Cytoline<sup>TM</sup> 1 beads. If the bed is fluidized at a velocity higher than that of the sedimentation rate, then the microcarriers will be carried to the top of the reactor. This is known as flush out, and usually occurs when the media is run through the bed at velocities exceeding 10 times the minimum fluidization velocity. The sedimentation rate for the Cytoline<sup>TM</sup> 1 microcarriers is 120-220 cm/min of media, dictated by the Cytoline<sup>TM</sup> 1 microcarrier product information brochure. To avoid flush out and to achieve optimal operation, the fluidized bed bioreactor will flow media at about 1.9 times the minimum fluidization velocity for the average sized Cytoline<sup>TM</sup> 1 bead bed, which corresponds to around 28,000L of media

per hour or a flow rate of approximately 19 cm/min. This value was determined by performing a sensitivity analysis upon the minimum fluidization rates for the bead size distribution within the reactor. Calculations for the minimum fluidization velocity are shown in Appendix A. The fluidized bed reactor will operate at the minimum fluidization velocity of the largest particles, which is 1.9 times the minimum fluidization velocity for an average sized Cytoline™ 1 bead and about 6.2 times the minimum fluidization velocity of the smallest Cytoline™ 1 beads. The void fraction of the Cytoline™ 1 bed is estimated for an ellipsoidal packed bed as  $\varepsilon = 0.35$ , which is equivalent to near-perfect packing for ellipsoidal particles. The Cytoline™ 1 particles are ellipsoidal, and due to the gentle agitation before fluidization in the inoculation step, it is fair to assume that the beads will fall into the configuration for optimal packing. Additionally, edge effects from the sides of the fluidized bioreactor are considered to be negligible in comparison to the size of the bed because the effective diameter of the beads is on the order of millimeters, while the diameter of the bed is on the order of meters. More precisely, the equivalent spherical diameter of the Cytoline™ 1 particles is around 0.912 mm, while the effective diameter of the annular fluidized bed is about 1 meter. Therefore, we can negate the imperfections in packing due to the walls of the reactor. Perfect packing for smooth ellipsoidal particles corresponds to a void fraction of 0.31, but because the Cytoline™ 1 particles are porous, the void fraction is estimated to be slightly higher due to the uneven surface of the particle.

### 5.6.2. Nutrient Transport in Fluidized Microcarrier Culture

For microcarrier culture, the normalized gradient between the concentration of substrate in the local bulk medium and concentration of substrate at the cell surface is given by the expression found in Appendix B. For all substrate analysis, glucose was used as the nutrient under investigation. Glucose consumption inside the bioreactor is a good indication of cell growth and health—when cells are healthy and the exponential growth phase, glucose consumption is relatively simple to model. Experimentally, glucose assays are easy and non-cost intensive perform on effluent media samples. Lastly, for the purposes of this report, there are many well-documented mass transport parameters published for glucose, as opposed to other media components. When typical values were substituted for the parameters specific to glucose transport through cell culture media at

37°C, the normalized gradient between glucose concentration at the cell surface and in the bulk was at a value of approximately 0.01, which is far less than unity. Therefore, it is assumed in the fluidized bioreactor model with using microcarrier culture that there exists an adequate amount of mass transport between the microcarriers and the medium. It is noted that when the same analysis is applied to the packed bed reactor, the normalized gradient for glucose between the bulk and the cell surface concentrations is about 0.27, which is less than unity but non-negligible. This corresponds to the concentration of glucose at the cell surface to be 73% of the glucose concentration in the bulk media. All calculations and constants are given in Appendix B. Mass transfer between the microcarrier surface and the cell surface is generally not accounted for due to the fact that the microcarriers are designed in a manner that minimizes mass transfer resistance.

### **5.6.3. Fluidized Bed versus Packed Bed—Nutrient Composition Temporal Analysis**

In order to determine if fluidizing the bed was necessary, an analysis of cell growth profiles with fresh substrate influx was conducted, with mass balance equations and derivation shown in Appendix C. The novelty behind the fluidized bed reactor is the ability to sustain cell concentrations that are about two orders of magnitude greater than cell concentrations in traditional stirred tank cultures due to increased surface area and a nutrient-replenishing fluidizing stream. In order to determine the efficacy of fluidizing the replenishing nutrient stream, glucose consumption was examined inside the three different reactor conformations: a fluidized bed reactor, a packed bed reactor, and a batch reactor. If it is determined that the bed could be operated at a velocity that is smaller than the fluidization velocity determined in section 5.6.1—which would correspond to packed bed reactor operations—or without replenishing the nutrients in the reactor at all—corresponding to batch reactor operations—then the fluidization of the reactor would be unnecessary and wasteful in terms of media and energy requirements to operate the reactor. For the fluidized bed, the nutrient replenishing stream propagates through the reactor at the fluidization velocity dictated earlier in this section. The packed bed is characterized by an influx of glucose that is propagating slower than the minimum fluidization velocity for all particle sizes (chosen to be 4,430 L/hr). A batch reactor was also considered which had neither inflow nor outflow of glucose.

There are a few key assumptions that outline the validity of this model. The first concerns the contents of the reactor. The media is assumed to have 8 g/L of glucose initially and that the reactor has been charged initially with media. The cells are assumed to be in exponential growth phase, a protocol that was suggested from the GE microcarrier handbook. Because the bed is fluidized and the cells are on microcarriers, it is assumed that there is no notable cell loss from the reactor. In the same vein, there is no additional cell addition to the reactor other than the growth inside the reactor. Glucose consumption is also assumed to be solely due to the metabolic activity of the cells and not for product formation. Lastly, we are assuming that all reactor systems are well mixed such that there exists no spatial dependence on cell or substrate concentration inside the reactor at a given time point.

The results of this analysis are plotted and shown in Appendix C. The time interval considered in this analysis was the total run time of the fluidized bioreactor, 12.8 days. The cells were considered nutrient starved when the glucose concentration within the reactor was below 4 g/L. The batch reactor was considered nutrient starved after less than two days of operation. The cells in the packed bed reactor were considered nutrient starved after about 8 days of operation. The fluidized bioreactor maintained a glucose concentration of 4.8 g/L after the 12.8 day operating time and was therefore found to be able to sustain the cell culture.

#### **5.6.4. Fluidized Bed v. Packed Bed—Nutrient Consumption Spatial Analysis**

The glucose consumption and cell growth profiles were modeled for a packed bed to examine the spatial dependence of nutrient mass transfer to the cells. This analysis is conducted in order to determine if the reactor could be operated using a packed bed model as opposed to a fluidized bed model. Running the reactor in a packed bed confirmation would decrease media requirements and operational energy requirements, and would therefore be more advantageous from a financial standpoint. In the previous section, it was assumed that the concentration of glucose in the reactor was spatially independent with regards to the bulk concentration. While this is a good assumption for the fluidized bed reactor, it was shown earlier in this report that the normalized gradients between the bulk and cell surface concentrations in a packed bed bioreactor is non-negligible at 0.27. To further explore the spatial nutrient consumption in the packed

bioreactor, a simple model was implemented using the Transport of Diluted Species (chds) module of the Comsol Multiphysics Software. In order to execute the model, a few assumptions are made, like in the temporal analysis of the reactors. The media is assumed to have 8 g/L of glucose initially, which corresponds to  $44.406 \text{ mol/m}^3$  of glucose, in the reactor. It is also assumed the reactor is initially charged with media. The cells are assumed to be in exponential growth phase, a protocol that was suggested from the GE Microcarrier Handbook. Because the bed is fluidized and the cells are on microcarriers, it is assumed that there is no notable cell loss from the reactor. Furthermore, there is no additional cell addition to the reactor other than the growth inside the reactor. Glucose consumption is also assumed to be solely due to the metabolic activity of the cells and not for product formation.

Results from this analysis are plotted in Appendix D. After 60 hours of operation, the concentration of glucose over most of the packed bed has dropped to about 20% of its original value, creating a nutrient deprived environment in over half of the bed. After 72 hours, the cells at the bottom of the packed bed fully consume the glucose in the media before it can get to any other cell layer in the bed, leaving the majority of the bed nutrient deprived. After 72 hours, the cells are completely nutrient deprived over the majority of the bed and would most certainly be dead. Time points shown were determined to show when the reactor would be fully nutrient deprived and therefore unable to sustain cell growth. In order to meet the monoclonal antibody production goals, the packed bed reactor would need to operate for 306 hours, which using the model presented, would lead to widespread cell death over the majority of the bed. Therefore, fluidization of the microcarrier bed is necessary in order to meet the monoclonal antibody production goals.

## 6. Purification Process Description

After the upstream process, the extracellularly secreted product must be separated from the media solution and purified according to FDA standards. Furthermore, each batch of downstream purification will be quality checked to ensure quality standards are met and that the generic drug matches the brand name formulation of Ritxan<sup>®</sup>. All following descriptions apply to both processes unless otherwise specified.

## **6.1. Buffer Preparation**

Many downstream operations, such as the chromatography columns, will require various buffers for each batch. Buffers will be purchased from GE Healthcare and mixed with WFI in disposable containers. Disposable containers will help minimize cross-contamination and eliminate any cleaning and sterilization procedures. Once prepared, the pH and conductivities of the buffers will be checked and controlled until the buffers are used. If the buffers are improperly prepared, they will have to be altered to reach the proper pH.

## **6.2. Holding Tank**

For both cases, a large amount of spent media solution (20,000L or more) containing product will be recovered at the end of the production step. However, downstream purification must be done in smaller batches to accommodate the volume capacity of the Protein A column.

### **6.2.1. Stirred Tank Bioreactor Process**

For the stirred tank process, the cells will be transferred via a peristaltic pump into a holding tank for eventual purification. Then, the approximately 20,000L solution of cells, media, and product will proceed downstream in ten batches of 2,000L each.

### **6.2.2. Fluidized Bioreactor Process**

Unlike the traditional stirred process, the fluidized bioreactor process will be continuous. Therefore, spent media will be continuously collected from the top at a rate of 19 cm/min. Approximately 5.7 million liters of spent media and product will have to be collected and stored in fifty-eight 50,000L storage tanks. From these tanks, Protein A purification will be conducted in two batches. The eluted product will then be processed further downstream in two batches.

## **6.3. Microcarrier and Cell Disposal**

As a part of downstream processes, after the media solution (containing product) has been removed from the fluidized bioreactor, the cells and microcarriers must be disposed appropriately. Per batch, 730L of microcarriers and cells will be heat treated to 250°C in order to kill the cells. Then, the microcarriers and cells will be disposed according to FDA regulations.

#### **6.4. Centrifugation**

For the stirred tank bioreactor process, contents of the holding tank are transferred into a centrifuge by a peristaltic pump in smaller batch sizes of 10,000L per batch. The centrifuge helps remove the product and other components suspended in the media solution from the solid biomass and other waste. The centrifuge will operate at a throughput rate of 10,000 L/hr. The centrifuge is operated at 25°C and has a yield of 98% product with about 2% of desired product being discarded in the solid waste stream. Centrifugation for stirred tank batches will require a total time of 6 hours including CIP and SIP.

#### **6.5. Tangential Flow Filtration**

For the fluidized bioreactor process, the media will not have to be separated from the cells due to the nature of the setup. Therefore, tangential flow filtration offers a more efficient and cost-effective method to perform a primary purification step. TFF helps filter large molecular weight aggregates, large proteins, and any remaining cells. The feed (media containing product and other particles) is passed tangentially over a membrane in order to prevent filter caking. This will be a continuous process but six TFF filters will be used in parallel to help reduce the individual operational time of the unit. The feed will be filtered at a speed of 5 L/min/m<sup>2</sup> and each filter membrane will have a cross sectional area of 1.8m<sup>2</sup>. In the case of the fluidized bioreactor process, the filtration step will require 58 TFF units with a cross sectional area of 3.0m<sup>2</sup> and about 4.5 days to process the media output of the bioreactor and have a 98% yield.

#### **6.6. Protein A Chromatography**

Protein A Chromatography is the first chromatography column in the downstream process. It acts as the first main isolation step for product and isolates the monoclonal antibody via affinity chromatography. Therefore, the column is useful in eliminating host cell DNA, media components and endogenous viral particles.

After centrifugation (for the stirred tank bioreactor process) or after tangential flow filtration (for the fluidized bioreactor process), the supernatant pH is equilibrated to 7.0 using H<sub>3</sub>PO<sub>4</sub>. Then, the solution is directly transferred to the Protein A column via a peristaltic pump. Before the first batch, the column must be loaded with resin. Then, at the beginning of each batch, the column must be equilibrated with 2-3 bed volumes of equilibration buffer



(20mM sodium phosphate at pH 7.0). The supernatant from the centrifugation step is then directly applied to the column. Then, an intermediate wash step is used with wash buffer (containing 0.25M TMAC or 0.25M TEAC) at a pH of 6. Finally, the monoclonal antibody product is eluted from the column by washing with elution buffer (25mM sodium citrate) at a pH of 2.8. The low pH allowed for the removal of the product from the resin so it could be collected in the elution stream. The elution stream will be transferred into a storage tank. Finally, the column is then regenerated with 2-3 bed volumes of regeneration buffer. The maximum linear flow rate for the column will be about 400 cm/hr. The Protein A column is expected to have about 98% product yield. For the stirred tank process, it will require a total of 1.5 days to process all input (each input refers to a batch of 2000L of solution). For the fluidized bioreactor process, a linear flow rate of 40 cm/min will be used and require 4.5 days to process all media input (where each input refers to half of the media output of the fluidized bioreactor). These times also account for the sterilization required in between each batch.

### **6.7. Cation Exchange Chromatography**

Cation exchange chromatography is used to reduce high molecular weight aggregates, charge variants, residual DNA and host cell proteins. Cation exchange uses positively charged resin to bind to negatively charged particles (including the product) while positive and neutral particles pass through the column.

Before the first batch, the column must be loaded with resin. Then, before each batch, the column must be equilibrated with a buffer (60nM NaCl) at a pH of 5.5 and conductivity of 6.5 mS/cm. Then, about 2000L from the holding tank after the Protein A step will be transferred into the column from both processes. The first wash buffer is salt free and has a pH between 7.0 and 7.8 and conductivity between 0.2 and 2 mS/cm. A second buffer wash is performed at pH 5.5 and conductivity between 0.5 and 3.0 mS/cm. Finally, the column is eluted to remove the desired product from the resin using elution buffer (160-175mM NaCl) at pH 5.5 and a conductivity of 12-20 mS/cm. The eluted stream is transferred to a storage tank. Finally, the column is regenerated using regeneration buffer. The maximum linear flow rate for the column will be about 400 cm/hr. The column is expected to have about 98% product yield. Including sterilization in between batches, it will require about 1-1.5 days to process the input into the columns for both processes.

### **6.8. Anion Exchange Chromatography**

Anion exchange chromatography is also used to reduce high molecular weight aggregates, charge variants, residual DNA and host cell proteins. Anion exchange uses negatively charged resin to bind to positively charged particles while negative particles (such as the product) pass through the column. Before each batch, the column must be equilibrated with equilibration buffer (20-100mM NaCl) at pH 8.0. The media solution is applied to the column in batch sizes of 2000L for both processes. Then, the column is washed with buffer. The product is removed in the wash step and stored in a storage tank. Finally, the column is washed with elution buffer (1M NaCl) at pH 8.0. Then, the column is regenerated using regeneration buffer. The maximum linear flow rate for the column will be about 400 cm/hr. The column is expected to have about 98% product yield. Including sterilization in between batches, it will require about 1-1.5 days to process all batches for both processes.

### **6.9. Viral Nanofiltration**

Viral nanofiltration will be used after the anion exchange chromatography step to filter the viruses. Viral nanofiltration is a size-based membrane separation in which proteins can easily pass through the hollow fiber walls of the membrane while viruses are captured within the pores. This type of filtration is dependent upon pressure, flow rate, protein concentration, and the ratio of product volume to membrane surface area. First, the filters and unit must be flushed thoroughly with WFI. Then, the unit must be equilibrated with equilibration buffer. Finally, the sample (in sizes of 2000L for each process) can be loaded and the permeate will be collected for further purification. Its recovery rate of monoclonal IgG at 20mg/ml product concentration is about 99%. With six units running in parallel, this process will require about 6 hours for both processes.

### **6.10. Ultrafiltration**

After viral inactivation, size-based ultrafiltration is used to remove small viral and foreign particles. A filter with a 100kD MWCO will be used to keep the desire product in the retentate but allow for smaller particles to be removed. This step will also help concentrate the product to a final concentration of about 10 mg/mL. To help decrease the process time for the filtration step, six filters will be run in parallel. The process will require about 6 hours

where each batch size equals 2000 L for both processes. Its recovery rate of monoclonal antibody will be about 96%.

### **6.11. Sterile Filtration**

Sterile filtration serves as the last purification step to help remove any final endotoxins and also to help concentrate the product to the appropriate concentration. To help decrease the process time for the filtration step, six filters will be run in parallel. The process will require about 6 hours to process all input (where the input for each process is about 2000L). The recovery rate is expected to be about 96%.

## **7. Major Unit Operation Specifications**

### **7.1. Common Units**

#### **7.1.1. Pumps**

A peristaltic pump is used to transfer fluid from throughout the process as depicted by the pump icon in the process flow diagrams. The pump can be purchased from Watson Marlow and is the 825 Peristaltic Pump. In both the stirred tank and fluidized bioreactor processes, the power of the pump ranges from 1.5-5.0 hp. The pumps operate at room temperature and a 50 psi (3.5 bar) pressure drop with maximum flow rate of 33.3 L/min. The pump will be sterilized and are purchased from Watson Marlow for the cost of \$13,000 per pump. The same company also offers a variety of pumps with maximum flow rates of ~300L/min which will be used for the fluidized bioreactor process. Watson Marlow Bioprene sterile tubing is used to transfer the fluid through the variety of processes.

#### **7.1.2. Media Sterilization Filters**

The 0.2 micron filter is used to purify the media by removing any impurities and toxins before introduction to the bioreactors. The filter is 30 inches in size and the filtration area is 1.8m<sup>2</sup> (18ft<sup>2</sup>). The membrane filter material is Polyethersulfone (PES) and operates at room temperature with a max differential pressure of 0.5 bar. The filters require a type of housing for security that requires a one-time purchase from Sartorius Stedim Biotech for the cost of \$2,000. The filters are disposable and replaced after every

batch. They are purchased from Sartorius Stedim Biotech under the model name Sartopore 2 for the cost of \$650/unit. The filters are disposable so no CIP or SIP will be required, but SIP is required for the housing unit.

### **7.1.3. Digital Control Unit**

This system is used to control agitation speeds, temperature at 37°C, pH between 7.0-7.2 and dissolved oxygen at a specified level for all bioreactors in both processes. The control units are \$300 per unit and are purchased from Vernier Software and Technology. Each unit will control one parameter. A total of 24 control units will be needed for all the bioreactors.

## **7.2. Inoculum Prep**

### **7.2.1. Bag Bioreactor (PFD 01/P-02)**

The WAVE™ Bioreactor created by GE Life Sciences will be used for the bag bioreactors. The WAVE™ Bioreactor 2/10EH – electric rocker base comes equipped with integrated temperature control and aeration pump. It is an efficient and cost-effective method for expansion of CHO cells in suspension. The rocking action of the base provides continual mixing and oxygen transfer. For each batch, a new pre-sterilized cell bag can be used. The system requires no cleaning or sterilization and helps eliminate cross-contamination between batches. Each bioreactor unit for this stage of the process is able to hold one 200mL bag. The unit is purchased from GE Healthcare for \$16,480. The bags are plastic pre-sterilized, single use chambers for non-invasive mixing of fluids using the WAVE™ rocker. The unit is kept at 37°C, 1 bar, a pH ranging from 7.0-7.2, and 50% DO. The process time for this unit is 3.5 days. The 20 disposable bags have sensors and ports for oxygen and media and are purchased from GE Healthcare for \$202.00 per bag.

## **7.3. Seed Bioreactor Section (Stirred Tank Process)**

### **7.3.1. Storage/Mixing Tank (PFD 01/P-03)**

This tank is used to store and mix powder serum-free media with water for injection before being filtered and then transferred into the first seed bioreactor. The tank will be manufactured from Stainless Steel 316L, has a volume of 150L and a working

capacity of 70%. It stands 0.9m tall and is 0.4m in diameter. For sterilization, CIP and SIP will be conducted after each batch. The tank operates at room temperature and at 1 bar. The tank is manufactured by Sharpsville Container and costs \$110,000.

### **7.3.2. Seed Bioreactor 1 (PFD 01/P-07)**

The first seed bioreactor is the disposable WAVE™ 500/1000 bioreactor purchased from GE Healthcare. The unit consists of a permanent base used for rocking along with disposable cell culture bags. It is stainless steel with dimensions of 201x124x160 cm. Each bioreactor unit for this stage of the process is able to hold one 200 L bag. The unit is the GE WAVE™ Bioreactor purchased from GE Healthcare for \$404,500. The bag has a 200L volume with 50% working volume. The 200L bags are plastic pre-sterilized, single use chambers for non-invasive mixing of fluids using the WAVE™ rocker. The unit is kept at 37°C, 1 bar, a pH ranging from 7.0-7.2, and 50% DO. The pH, temperature, and oxygen levels will be controlled with PID controls. The process time for this unit is 4 days. The plastic disposable bags have sensors and ports for oxygen and media and are purchased from GE Healthcare for \$1,200 per bag.

### **7.3.3. Storage/Mixing Tank (PFD 01/P-09)**

This tank is used to store and mix powder serum-free media with water for injection before being filtered and then transferred into the second seed bioreactor. The tank will be manufactured from Stainless Steel 316L, has a volume of 1,500L and a working capacity of 70%. Its height is 2.3m and its diameter 0.9m. For sterilization, CIP and SIP will be conducted after each batch. The tank operates at room temperature and at 1 bar. The tank is manufactured by Sharpsville Container and costs \$120,000.

### **7.3.4. Seed Bioreactor 2 (PFD 01/P-13)**

The second seed bioreactor is another disposable bag bioreactor from GE Healthcare. The unit is the XDR-50 to 2000 Single Use Bioreactor and provides use with working culture volume up to 2,000L. The unit consists of a permanent base used for rocking along with disposable cell culture bags. Each bioreactor unit for this stage of the process is able to hold one 2,000L bag. The unit is purchased from GE Healthcare for \$600,000. The bag has a 2,000L volume with 50% working volume. The 2000 L bags are plastic pre-sterilized, single use chambers for non-invasive mixing of fluids using the

WAVE™ rocker. The unit is kept at 37°C, 1 bar, a pH ranging from 7.0-7.2, and 50% DO. The pH, temperature, and oxygen levels will be controlled with PID controls. The process time for this unit is 4 days. The plastic disposable bags have sensors and ports for oxygen and media and are purchased from GE Healthcare for \$1,800 per bag.

## **7.4. Seed Bioreactor Section (Fluidized Process)**

### **7.4.1. Storage/Mixing Tank (PFD-03/P-03)**

This tank is used to store and mix powder serum-free media with water for injection before being filtered and then transferred into the first seed bioreactor. The tank will be manufactured from Stainless Steel 316L, has a volume of 150L and a working capacity of 70%. It stands 0.9m tall and is 0.4m in diameter. For sterilization, CIP and SIP will be conducted after each batch. The tank operates at room temperature and at 1 bar. The tank is manufactured by Sharpsville Container and costs \$110,000. The outflow from the tank will be split into three equal streams into three seed bioreactors running in parallel.

### **7.4.2. Seed Bioreactors in Parallel (PFD 03/P-07)**

The first set of seed bioreactors involves three disposable bag bioreactors in parallel. Each has a 70L bag volume with a 33.3L working volume. The bioreactors are the disposable WAVE™ 500/1000 bioreactor purchased from GE Healthcare. The unit consists of a permanent base used for rocking along with disposable cell culture bags. It is stainless steel with dimensions of 201x124x160 cm. Each bioreactor unit for this stage of the process is able to hold one 70L bag. The unit is the GE WAVE™ Bioreactor purchased from GE Healthcare for \$404,500. The bag has a 70L volume with 50% working volume. The unit is kept at 37°C, 1 bar, a pH ranging from 7.0-7.2, and 50% DO. The pH, temperature, and oxygen levels will be controlled with PID controls. The process time for this unit is 5 days. The plastic disposable bags have sensors and ports for oxygen and media and are purchased from GE Healthcare for \$1,000 per bag.

### **7.4.3. Storage/Mixing Tank (PFD 03/P-09)**

This tank is used to store and mix powder serum-free media with water for injection before being filtered and then transferred into the second seed bioreactor. The

tank will be manufactured from Stainless Steel 316L, has a volume of 1,500L and a working capacity of 70%. Its height is 2.3m and its diameter 0.9m. For sterilization, CIP and SIP will be conducted after each batch. The tank operates at room temperature and at 1 bar. The tank is manufactured by Sharpsville Container and costs \$120,000.

#### **7.4.4. Second Seed Bioreactor (PFD 03/ P-13)**

The second seed bioreactor is another disposable bag bioreactor from GE Healthcare. The unit is the XDR-50 to 2000 Single Use Bioreactor and provides use with working culture volume up to 2,000L. The unit consists of a permanent base used for rocking along with disposable cell culture bags. Each bioreactor unit for this stage of the process is able to hold one 2,000L bag. The unit is purchased from GE Healthcare for \$600,000. The bag has a 2,000L volume with 50% working volume. The 2000L bags are plastic, pre-sterilized, single use chambers for non-invasive mixing of fluids using the WAVE™ rocker. The unit is kept at 37°C, 1 bar, a pH ranging from 7.0-7.2, and 50% DO. The pH, temperature, and oxygen levels will be controlled with PID controls. The process time for this unit is 5.5 days. The plastic disposable bags have sensors and ports for oxygen and media and are purchased from GE Healthcare for \$1,800 per bag.

### **7.5. Production Section**

#### **7.5.1. Storage/Mixing Tank (PFD 01/ P-15 & P-19)**

This tank is used to store and mix powder serum-free media with water for injection before being filtered and then transferred into the third tank bioreactor. The tank will be manufactured from Stainless Steel 316L, has a volume of 10,000L and a working capacity of 70%. Its height is 3.5m and its diameter is 2.0m. For sterilization, CIP and SIP will be conducted after each batch. The tank operates at room temperature and at 1 bar. The tank is manufactured by Sharpsville Container and costs \$130,000. There are two of these media storage tanks for a total of 20,000L.

#### **7.5.2. Stirred Tank Bioreactor (PFD 01/ P-23)**

The production stirred tank bioreactor is a stainless steel jacketed bioreactor purchased from Techniserv Inc (SCAL-TECH Products). It has a 25,000L volume with a height of 5.0m and a diameter of 2.5m. Following each batch, CIP and SIP is used to

sterilize the unit. The pH, temperature and oxygen levels will be controlled with PID controls and will run for 16 days. The bioreactor operates at 37°C and about 1.5-2 bar. The bioreactor will be bought for \$900,000.

#### **7.5.3. Storage/Mixing Tank (for fluidized bioreactor) (PFD 03/ P-15 & P-19)**

This tank is used to store and mix powder serum-free media with water for injection before being filtered and then transferred into the last holding tank. As soon as media is mixed, it will be filtered and fed to the following storage tank before a new batch of media is mixed. This process will continue in order to meet the media requirement for the fluidized bioreactor. The tank will be manufactured from Stainless Steel 316L, has a volume of 10,000L and a working capacity of 70%. Its height is 3.5m and its diameter is 2.0m. For sterilization, CIP and SIP will be conducted after each batch. The tank operates at room temperature and at 1 bar. The tank is manufactured by Sharpsville Container and costs \$130,000.

(Note: There are two of these media storage tanks for a total of 20,000L.)

#### **7.5.4. Media Storage Tank (for fluidized bioreactor) (PFD 03/ P-23)**

This tank is used to store the media before it enters the fluidized bioreactor. This unit has a volume of 50,000L, with a height of 6.0m and a diameter of 3.25m. Media from the mixing tanks (PFD 03/ P-15 & P-19) is fed to this storage tank and the fed to the fluidized bioreactor. As media is being transferred from the storage tank to the bioreactor, media from the mixing tanks is also being transferred to the storage tank. As previously stated, this continuous media transfer process is used in order to meet the total media requirement of 5.7 million liters for the fluidized bioreactor. For sterilization, CIP and SIP will be conducted after each batch. It will be purchased from Sharpsville Container for a cost of \$165,000.

#### **7.5.5. Fluidized Bioreactor (PFD 03/P-25)**

The fluidized bioreactor is the GE Healthcare Cytopilot™ Production Bioreactor. It has a 25,000L volume and annular shape. Following each batch, CIP and SIP is used to sterilize the unit. The pH, temperature and oxygen levels will be controlled with PID controls and will run for 13 days. The bioreactor operates at 37°C, about 1 bar, and DO of 30%. The bioreactor will be bought for \$1,500,000.



## **7.6. Downstream Purification**

### **7.6.1. Holding Tank (Stirred Tank Process) (PFD-03/P-25)**

The holding tank is used to store the output from the production process so that smaller batches can be sent downstream for purification. The unit has a volume of 25,000L with 80% capacity. It has a height of 5.0m and a diameter of 2.5m. For sterilization, CIP and SIP will be conducted after each batch. It will be purchased from Sharpville Container for a cost of \$145,000.

### **7.6.2. Holding Tank (Fluidized Process) (PFD-04/P-27)**

The holding tank is used to store the output for the production process so that smaller batches can be sent downstream for purification. It has a volume of 50,000L with 80% capacity. The tank has height of 6.0m and a diameter of 3.25m. The material is Stainless Steel 316L with an electro-polished finish. It will be purchased from Sharpville Container and costs \$165,000.

### **7.6.3. Tangential Flow Filtration Unit (PFD 04/ P-29)**

In the fluidized bioreactor process, the contents of the production bioreactor must be separated and the TFF unit allows the product or feed to flow directly tangentially along the surface of the membrane. The unit is purchased from Pall Corporation and each unit costs \$500,000. The model is the Cadence Single Pass TFF System and is Stainless Steel 316L with an electro-polished finish. It operates between 4-40°C, from 4-6 bar and pH between 2-13. The flow range is between 6-1000 L/h.

### **7.6.4. TFF Holding Tank (PFD 04/ P-31)**

The holding tank is used to store the output from the TFF unit so that batches can be sent downstream for purification. The unit has a height of 6.0m, a diameter of 3.25m, and a volume of 50,000L with 80% capacity. It will be purchased for \$165,000 from Sharpville Container.

### **7.6.5. Centrifuge (PFD 02/P-27)**

In the stirred tank bioreactor process, the contents of the production bioreactor must be separated into a solid waste stream and liquid stream containing the desired product. A hermetic disc-stack centrifuge produced by Alfa Laval is used. The

Culturefuge 400 model, constructed with Stainless Steel 316L, can be purchased from Alfa Laval for \$480,000. The centrifuge's throughput flow rate is 10,000 L/h. The unit operates at 25°C. CIP and SIP is used to sterilize the unit after each batch.

#### **7.6.6. Centrifuge Holding Tank (PFD 02/P-29)**

The centrifuge holding tank is used to store the output from the centrifuge unit so that batches can be sent downstream for purification. The unit has a height of 5.0m, a diameter of 2.5m, and a volume of 25,000L with 80% capacity. For sterilization, CIP and SIP will be conducted after each batch. It will be purchased from Sharpsville Container for a cost of \$145,000.

#### **7.6.7. Protein A Chromatography Column (PFD 02 & 04/P-31 & P-33)**

The Protein A affinity chromatography column is used to further purify the mainstream fluid to help isolate the product. The Chromaflow 2000/100-300 column will be purchased from GE Healthcare for \$200,000. The column is made of Stainless Steel 316L. The column is packed with nProtein A Sepharose 4 Fast Flow resin (distributed by GE Healthcare). The bed (portion of column occupied by resin) will be sized at a bed height of 0.5m and diameter of 2.54m. The bed volume is 2000L. The resin has a binding capacity of 50 mg/ml. The resin has a working velocity of 30-400 cm/h. The resin will flow through the column at 40 cm/min for the fluidized bed. The column is operated at 4°C, at 1 bar. The cost of the column is \$200,000 and the resin is \$8,000 per liter.

#### **7.6.8. Protein A Holding Tank (PFD 02 & 04 / P-33 & P-35)**

The Protein A holding tank is used to store the output from the Protein A column. The unit has a height of 3.0m, a diameter of 1.5m, and a volume of 5,000L with 80% capacity. For sterilization, CIP and SIP will be conducted after each batch. It will be purchased from Sharpsville Container for a cost of \$122,000.

#### **7.6.9. Cation Exchange Chromatography Column (PFD 02 & 04/ P-35 & P-37)**

The cation exchange chromatography column is used to further purify the mainstream fluid to help isolate the product. The Chromaflow 1000 column will be purchased from GE Healthcare for \$250,000. The column is made of Stainless Steel 316L. The column is packed with Eshmuno S resin (distributed by EMD Millipore). The

bed (portion of column occupied by resin) will be sized at a height of 0.5m and diameter of 2.54m. The resin has a binding capacity of greater than 60 mg antibody/mL media and a working velocity of up to 800 cm/hr. The column is operated at 25°C, at 1 bar. The cost of the column is \$250,000 and the resin is \$1,500 per liter.

#### **7.6.10. Cation Exchange Holding Tank (PFD 02 & 04 / P-37 & P-39)**

The cation exchange holding tank is used to hold and mix product from cation exchange chromatography with buffer solutions in preparation for anion exchange chromatography. The unit has a height of 3.0m, a diameter of 1.5m, and a volume of 5,000L with 80% capacity. For sterilization, CIP and SIP will be conducted after each batch. It will be purchased from Sharpsville Container for a cost of \$122,000.

#### **7.6.11. Anion Exchange Chromatography Column (PFD 02 & 04/ P-39 & P-41)**

The anion exchange chromatography column is used to further purify the mainstream fluid to help isolate the product. The Chromaflow 1000 column will be purchased from GE Healthcare. The column is made of Stainless Steel 316L electropolished. The column is packed with Capto Q resin (GE Healthcare). The bed (portion of column occupied by resin) will be sized at a height of 0.5m and diameter of 2.54m. The bed volume is 2,000L. The resin has a binding capacity of greater than 100 mg antibody/mL medium and a working velocity of 700 cm/hr. The column is operated at 25°C, at 1 bar. The cost of the column is \$250,000 and the resin is \$1,800 per liter.

#### **7.6.12. Anion Exchange Holding Tank (PFD 02 & 04/ P-41 & P-43)**

The anion exchange holding tank is used to collect the product from the anion exchange column. The unit has a height of 5.0m, a diameter of 2.5m, and a volume of 25,000L with 80% capacity. For sterilization, CIP and SIP will be conducted after each batch. It will be purchased from Sharpsville Container for a cost of \$145,000.

#### **7.6.13. Viral Nanofiltration Unit (PFD 02 & 04/ P-43& P-45)**

The viral filtration unit will be purchased from AsahiKasei Bioprocess. The Planova BioEX membrane is effective against enveloped and non-enveloped viruses as small as 18nm while allowing proteins as large as 200kD to pass through without denaturing. This membrane is ideal for our 145kD product. The unit consists of a

permanent virus filtration controller along with disposable filters. The unit can handle up to 5000L of volume at for each batch run. The MW cutoff for this filter is 200kD and the protein throughput is 6.5 kg/m<sup>2</sup> per 3 hours. The filter effective surface area is 1.8m<sup>2</sup> and the suggested operating pressure is less than 343 kPa. The filter housing can be purchased from AsahiKasei Bioprocess for \$15,000 and will be SIP sterilized. The membrane filters are disposable and cost \$2,000 per filter.

#### **7.6.14. Ultrafiltration Unit (PFD 02 & 04/ P-45 & P-47)**

The ultrafiltration unit will be purchased from Pall Corporation. The membrane has a MW cutoff of 100kD. The unit consists of a permanent base along with disposable filters. The material of the disc filters is Omega Polyethersulfone and the filter has an effective surface area of 1.8m<sup>2</sup>. The housing unit will be sterilized through SIP. The filter housing and membranes are purchased from Pall Corporation and cost 12,000 per housing unit and \$1,000 per filter membrane.

#### **7.6.15. Final Sterile Filtration Unit (PFD 02 & 04/ P-47 & P-49)**

The final sterile filtration unit will be purchased from Sartorius Biotech. The Sartopore 2 membrane has a pore size of 0.2 micron and the filter material is Polyethersulfone. The filtration area is 1.8m<sup>2</sup> and the max differential pressure is 5 bar at 20°C. The unit consists of a permanent base along with disposable filters. The housing membrane unit sterilization is SIP and the membranes are disposable. The housing unit costs \$2,000 and the filter membranes cost \$650/unit.

### **7.7. Additional Downstream Separation (Fluidized Bioreactor Process)**

#### **7.7.1. High Temperature Inactivation (PFD 05/ P-52)**

An industrial size autoclave can be purchased from WSF Industries Inc. This unit serves to kill the cells attached to the microcarriers in order to allow for safe disposal. The unit is made of Stainless Steel 316L with an electro-polished finish. For sterilization, CIP and SIP will be conducted after each batch. The autoclave will operate at 25°C. The cost of this unit is \$500,000.

## **8. Additional Equipment Description**

Additional costs will need to be considered for equipment not depicted in the process flow diagrams. These items help maintain good manufacturing practices. Since we are working at a refurbished green site, the site is equipped with many of these individual operations and processes so some of the equipment expenses are added to the annual expenses. Individual prices for the equipment are not needed because it does not have to be bought. Some equipment will have to be bought and the price will be included. A summary of these costs can be found in Section 10.

### **8.1. Biosafety Cabinet**

Three biosafety cabinets will be required to provide a sterile environment to handle and transfer the vial of cells into the bag bioreactors in the inoculum prep section. The biosafety cabinet is manufactured by Thermo Scientific<sup>TM</sup>. The biosafety cabinets will already be in the plant and the costs will be included in the annual expenses.

### **8.2. Cell Bank**

Though out of scope of this project, a cell bank will be required to provide the initial inoculum for the inoculum prep sections. The cell bank will store optimized recombinant CHO cells designed to produce rituximab and be created in a clean lab. These cells will be cultured and grown to a final cell density of  $1 \times 10^8$  cells/mL and aliquoted into 2mL vials. Before either facility begins operation, a cell bank will be created to sustain the inoculum requirements with more cells being added to the cell bank as needed.

### **8.3. Air Generator (HVAC Equipment)**

Purified air will be the source of oxygen throughout the process and will be provided by an HVAC system. The system will supply clean air to bioreactors, clean rooms, and other process equipment. The air generator will be in the plant and the costs will be included in the annual expenses.

### **8.4. Clean Steam Generator**

The clean steam generator is used to produce steam from WFI for SIP procedures. The clean steam generator will already be in the plant and the costs will be included in the annual expenses.

### **8.5. CIP Skids**

Clean-In-Place (CIP) is a technique used to clean equipment. A CIP skid is portable and contains the cleanings solutions and equipment necessary. In order to clean all the unit operations and storage tanks, ten CIP skids will be needed for this process. They will be manufactured by Sani-matic and each will cost about \$100,000.

### **8.6. Buffer Transfer Bags**

Buffer transfer bags are used to transfer media or buffer to appropriate unit operations. These disposable bags and bag holders are manufactured by HyClone and will cost approximately \$70,000 a year.

### **8.7. Filter Integrity Tester**

Though disposable filters are used throughout the process, they must be tested before each use to assure they are not clogged, torn or general unusable. The filter integrity tester from Millipore and is in the plant so the costs will be included in the annual expenses.

### **8.8. Tube Fuser and Sealer**

At various times in the process, a tube fuser will be necessary to connect tubing between disposable and permanent process equipment such as buffer transfer steps or filtration steps. A tube sealer is also necessary to seal any plastic tubing to prevent leaks in bags during transport. The Sartorius Stedim Biotech's Sterile Tube Fuser is a fully automatic device that cuts and fuses tube ends together to ensure sterility. The tube fuser and sealer are in the plant and the costs will be included in the annual expenses.

### **8.9. Water Treatment Package**

Though WFI will be used for most of the water requirements in the process, U.S. Pharmacopeia (USP) grade water is needed for temperature control needs in the bioreactor process. This water will not be in contact with the cell culture suspension, but inserted into the stainless steel bioreactor jackets for cooling. The cost for the water treatment package is included in the annual expenses.

### **8.10. WFI Still**

The WFI still is used to purify water produced for the water treatment package for use with cell culture. WFI is used to ensure sterility of not only the product but also of the process equipment to help eliminate cross-contamination issues. The still is manufactured by the Paul Mueller Co. The WFI still will already be in the plant and the costs will be included in the annual expenses.

### **8.11. Biowaste and Neutralization Tanks**

All biowaste produced in the process will have to be treated appropriately before disposal. All waste potentially containing live culture is sent to the biowaste tank. All other waste, such as used buffers, is sent directly to a neutralization tank. Approximately 178L of cells are coming out of the tank bioreactor process. The neutralization tank will need to be about 125,000L so five 25,000L tanks will be used. The tanks can be bought from Sharpsville Container for \$145,000 each.

A facility will be contracted out to help dispose of the non-liquid waste. This non-liquid waste includes the disposable WAVE™ bags and other disposable products. The disposable bags and all their parts are mainly made from plastics that are derived from petroleum. Current recycling concepts are focused on incineration in outsourced power plants to recover the energy from the petroleum. The costs of the outsourcing will also be added to the annual expenses.

### **8.12. Biowaste Inactivation System**

This system is needed to kill any live mass remaining in the biowaste tank. This includes all CIP and SIP washes, from the main bioreactors and primary recovery. The biowaste inactivation system is in the plant and costs will be included in the annual expenses.

### **8.13. Waste Neutralization System**

The waste neutralization system is needed to adjust the pH of cell-free waste to 7.0. Then, the waste can be disposed directly into the sewer line. This allows the wastes to be sent to the sewer. The waste neutralization system is in the plant and the costs will be included in the annual expenses.

#### **8.14. Quality Control Lab**

A quality control lab will review the quality of all factors involved in production. In the quality control lab, a set of procedures will be performed to ensure that the manufactured product adheres to a defined set of quality criteria. The costs of this lab will be included in the annual expenses.

#### **8.15. Laboratory Information Management System (LIMS)**

The Laboratory Information Management system (LIMS) is software that is used to manage a laboratory. It manages samples, users, instruments, and many laboratory functions. The LIMS unit will already be in the plant and the costs will be included in the annual expenses.

#### **8.16. Portable Pump on Cart**

In the case of pump malfunction, three extra pumps will be stored on portable carts as a replacement. An extra Watson-Marlow 825 pump and a cart will cost \$13,000 each.

#### **8.17. Refrigeration**

Freezing of the monoclonal antibody is necessary to keep the product stable for as long as possible. Effective temperatures for long term storage range from -20°C to -80°C. The freezing is done using an ultra cold freezer, which can reach temperatures up to -85°C. The freezer can hold up to 1050L (or 21 bags). Manufactured by Thermo Scientific™, the two freezers are a part of the plant and the costs are included in the annual expenses.

#### **8.18. Final Packaging**

The final output of the purification process will not have ready-to-administer product. The product will be shipped out to a facility that can add the proper fillers for storage and stability and ship the product to the necessary destinations. Final packaging, including both shipping and lyophilization, and will be performed by Quality BioResources, Inc. This process costs \$280,000 per batch.



## 9. Unit Specification Sheets

### 9.1. Stirred Tank Bioreactor Process

#### Bag Bioreactor (PFD 01/P-02)

<u>Description and Function</u>	To prepare the inoculum for seed bioreactor. An electric rocker base, with integral temperature control, weight controllers for perfusion culture, aeration pump, pH probe, and various rocking speeds is used along with 200mL Cellbag bioreactor chambers. The bags are plastic pre-sterilized, single-use chambers for non-invasive mixing of fluids using the WAVE™ rocker.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #01		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0.00524	0.0262
	Media	0.002	0
	Water	0.098	0.098
	Endotoxin	0	0
	Product	0	0
<u>Characteristics</u>	Model:	GE Healthcare WAVE™ Base 2/10EH	
	Construction:	High quality stainless steel and aluminum	
	Bag Bioreactor:	Cellbag 200mL Bioreactor Chamber	
	Sterilization:	Plastic Disposable Bags	
<u>Operations Conditions</u>	Temp:	37° C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	50%	
	Duration:	3.5 days	
<u>Purchase Cost</u>	Rocker and Control Unit:	\$ 16,480	
	200 mL Cellbag Bioreactor Chamber:	\$ 202.00 per bag (need 20)	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500	

**Media Prep (PFD 01/P-03)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #01

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Powder Media	0.2	Media Solution	98.00
	Water	97.8		

Characteristics

Material of Construction:	Stainless Steel 316L
Finish:	Electro-polish finish
Volume:	150L
Height:	0.9m
Diameter:	0.4m
Sterilization:	SIP/CIP

Operations Conditions

Temp:	25°C
Pressure:	1 bar

Purchase Cost

Storage Tank:	\$ 110,000
PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Seed Bioreactor (PFD 01/P-07)**

<u>Description and Function</u>	Bioreactor used for continued cell growth and production of monoclonal antibodies. The WAVE™ Bioreactor 500/1000 is a self-contained system with integral temperature control, aeration pump, and rocking controller for use with working culture volumes between 50L and 500L. The 200L bags are plastic pre-sterilized, single-use chambers for non-invasive mixing of fluids using the WAVE™ bioreactor.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #01		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0.52	3.04
	Media	2	0
	Water	98	98
	Endotoxin	0	0.81
	Product	0	0.02
<u>Characteristics</u>	Model:	GE Healthcare WAVE™ Bioreactor 500/1000	
	Construction:	Stainless Steel 316L	
	Finish:	Electro-polished	
	Dimensions:	201x124x160cm	
	Bag Bioreactor:	Cellbag 200L Bioreactor Chamber	
	Sterilization:	Plastic Disposable Bags	
<u>Operations Conditions</u>	Temp:	37° C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	50%	
	Duration:	4 days	
<u>Purchase Cost</u>	WAVE™ Bioreactor system:	\$ 404,500	
	200 L Cellbag Bioreactor Chamber:	\$ 1,200 per bag	
	PI control for Temp, pH, DO, Flow Rate, and Volume:	\$ 2,500/control = \$10,000	

**Media Prep (PFD 01/P-09)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #01

Operation Batch

<u>Materials Handled</u>	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
	Dry Media	18.00	Media	900.0
	Powder		Solution	
	Water	882.0		

<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	1500L
	Height:	2.3m
	Diameter:	0.9m
	Sterilization:	SIP/CIP

<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	1 bar

<u>Purchase Cost</u>	Storage Tank:	\$ 120,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Seed Bioreactor (PFD 01/P-13)**

<u>Description and Function</u>	Bioreactor used for continued cell growth and production of monoclonal antibodies. The XDR 2000 Single-Use Bioreactor offers a smooth transfer from development to manufacturing scale. It has robust industrial automation with an aeration pump. It provides exacting temperature control for use with working culture volumes up to 2000L. The 2000L bags are plastic pre-sterilized, single-use chambers for non-invasive mixing of fluids using the XDR bioreactor.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #01		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	3.04	15.21
	Media	20	0
	Water	980	980
	Endotoxin	0.81	4.06
	Product	0.02	0.16
<u>Characteristics</u>	Model:	XDR-2000 Single-Use Bioreactor	
	Construction:	Jacketed stainless steel vessel	
	Bag Bioreactor:	Cellbag 2000 L Bioreactor Chamber	
	Sterilization:	Plastic Disposable Bags	
<u>Operations Conditions</u>	Temp:	37° C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	50%	
	Duration:	4 days	
<u>Purchase Cost</u>	WAVE™ Bioreactor system:	\$ 600,000	
	2000 L Cellbag Bioreactor Chamber:	\$1,800 per bag	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500	

**Media Prep (PFD 01/P-15)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #01

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Media	190.0	Media	9500.0
	Powder		Solution	
	Water	9310.0		

Characteristics

Material of Construction:	Stainless Steel 316L
Finish:	Electro-polished
Volume:	10,000L
Height:	3.5m
Diameter:	2.0m
Sterilization:	SIP/CIP

Operations Conditions

Temp:	25°C
Pressure:	1 bar

Purchase Cost

Storage Tank:	\$ 130,000
PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Media Prep (PFD 01/P-19)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #01

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Media	190.0	Media	9500.0
	Powder		Solution	
	Water	9310.0		

Characteristics

Material of Construction:	Stainless Steel 316L
Finish:	Electro-polished
Volume:	10,000L
Height:	3.5m
Diameter:	2.0m
Sterilization:	SIP/CIP

Operations Conditions

Temp:	25°C
Pressure:	1 bar

Purchase Cost

Storage Tanks:	\$ 130,000
PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Production Bioreactor (PFD 01/P-23)**

<u>Description and Function</u>	Bioreactor used for continued cell growth and production of monoclonal antibodies. The final bioreactor serves to maximize product formation. Utilizes state-of-the-art bus technologies and supports process analytical technology (PAT).		
<u>Vendor</u>	Techniserv, Inc (SCAL-TECH Products)		
<u>PFD Reference</u>	PFD #01		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	6.73	356.32
	Media	400	0
	Water	19600	19600
	Endotoxin	4.056	36.34
	Product	0.16	9.41
<u>Characteristics</u>	Model:	SCAL-TECH Pro Suites Bioreactor	
	Finish:	Electro-polished	
	Volume:	25,000L	
	Height:	5.0m	
	Diameter:	2.5m	
	Sterilization:	SIP/CIP	
<u>Operations Conditions</u>	Temp:	37° C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	50%	
	Duration:	16 days	
<u>Purchase Cost</u>	Bioreactor:	\$900,000	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500	



**Media Prep Sterile Filtration (PFD 01/P-05, P-11, P-17, P-21)**

<u>Description and Function</u>	0.2 µm dead end filter to remove bacteria and other impurities from the serum free media prior to feeding the cultivators and production bioreactor. The sanitary stainless steel filter housings are designed for filtration applications. The filter capsules membranes are disposable ready-to-use filter or depth filter capsules.	
<u>Vendor</u>	Sartorius Stedim Biotech	
<u>PFD Reference</u>	PFD #01	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	Sartopore 2 0.2 µm
	Membrane Filter Material:	Polyethersulfone (PES)
	Size:	30"
	Filtration Area:	1.8m <sup>2</sup> (18ft <sup>2</sup> )
	Max. Differential Pressure:	5 bar (75 psi) at 20 °C
	Membrane Unit Sterilization:	SIP
	Membrane Sterilization:	Disposable
<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	0.5 bar (7.5 psi)
<u>Purchase Cost</u>	Filter Housing:	\$ 2,000
	Filter Membrane:	\$ 650/unit

**Pump (PFD 01 & 02/P-04, P-06, P-08, P-10, P-12, P-14, P-16, P-18, P-20, P-22, P-24, P-26, P-28, P-30, P-32, P-34, P-36, P-38, P-40, P-42, P-44, P-46, P-48)**

<u>Description and Function</u>	Pump to transfer fluid. High-flow hygienic pumps that are designed for low-shear sanitary pumping. They are ideal for viscous or shear sensitive products.	
<u>Vendor</u>	Watson-Marlow	
<u>PFD Reference</u>	PFD #01 and #02	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	825 Peristaltic Pump
	Material Construction:	304 Stainless Steel
	Flow Rate:	33.3 L/min
	Max Pressure:	3.5 bar
	Tubing:	Sterilized Bioprene, 40mm
	Sterilization	
<u>Operations Conditions</u>	Temp:	25°C
	Pressure Change:	50 psi (3.5 bar)
	Power:	1.5 – 5.0 hp
<u>Purchase Cost</u>	825 Peristaltic Pump:	\$13,000

**Storage Tank (PFD 02/P-25)**

Description and Function To store output from production bioreactor before being centrifuged.

Vendor Sharpsville Container

PFD Reference PFD #02

Operation Batch

<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	25,000 L
	Height:	5.0m
	Diameter:	2.5m
	Sterilization:	SIP/CIP

<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	1 bar

<u>Purchase Cost</u>	Storage Tank:	\$ 145,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Centrifuge (PFD 02/P-27)**

Description and Function To remove cells from the stream containing the monoclonal antibody product.

Vendor Alfa – Laval

PFD Reference PFD #02

Operation Batch

	<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
<u>Materials Handled</u> Cells	356.32	0
Media	0	0
Water	19600	19208
Endotoxin	36.34	33.44
Product	9.41	9.22

Characteristics

Model:	Culturefuge 400
Centrifuge Type:	Hermetic Disk Stack
Material of Construction:	Stainless Steel 316L
Finished:	Electro-polished product
Capacity Range:	5,000 to 20,000 L/hr
Sterilization:	SIP/CIP

Operations Conditions

Temp:	25°C
Throughput:	10,000 L/h

Purchase Cost \$ 480,000

**Centrifugation Pool Tank (PFD 02/P-29)**

Description and Function To store sterilized supernatant from the centrifugation process.

Vendor Sharpsville Container

PFD Reference PFD #02

Operation Batch

Characteristics

Material of Construction:	Stainless Steel 316L
Finish:	Electro-polished
Volume:	25,000L
Height:	5.0m
Diameter:	2.5m
Sterilization:	SIP/CIP

Operations Conditions

Temp:	25°C
Operating Pressure:	1 bar

Purchase Cost

Storage Tank:	\$ 145,000
PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Protein A Chromatography Column (PFD 02/P-31)**

<u>Description and Function</u>	To purify the antibody Fab fragments. It acts as the first main isolation step for product and isolates the monoclonal antibody via affinity chromatography. Therefore, the column is useful in eliminating host cell DNA, media components, and endogenous viral particles.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #02		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	<b>Cells</b>	0	0
	<b>Media</b>	0	0
	<b>Water Streams:</b>		
	Centrifuge effluent	19208	
	Equilibration buffer	2000	
	Wash buffer	2000	
	Elution buffer	4000	4000
	Regeneration buffer	6000	
	Protein A waste		29208
<u>Characteristics</u>	<b>Endotoxin</b>	33.44	20.06
	<b>Product</b>	9.22	9.04
	Model :	Chromaflow 2000/100-300	
	Column Diameter:	2.54m	
<u>Operations Conditions</u>	Bed Height:	0.4m	
	Material of Construction:	Stainless Steel 316L	
	Bed Volume:	2,000L	
	Resin	n-Protein A Sepharose 4	
	Binding Capacity:	50 mg antibody / ml media	
	Working Flow Velocity:	30-400 cm/h	
	Temperature Stability:	4-40°C	
<u>Purchase Cost</u>	Temp:	4°C	
	Pressure:	1.01 bar	
<u>Purchase Cost</u>	Column:	\$ 200,000	
	Resin:	\$ 8,000/ liters (need 2,000L)	

**Protein A Pool Tank (PFD 02/P-33)**

<u>Description and Function</u>	To collect product from anion exchange column.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #02	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	5,000L
	Height:	3.0m
	Diameter:	1.5m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp :	25°C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$ 122,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Cation Exchange Chromatography Column (PFD 02/P-35)**

Description and Function To purify and isolate antigen binding fragments from impurities.

Vendor GE Healthcare

PFD Reference PFD #02

Operation Batch

	Input (kg/Batch)	Output (kg/Batch)
<b>Cells</b>	0	0
<b>Media</b>	0	0
<b>Water Streams:</b>		
Prot A effluent	2000	
Cat wash 1 buffer	2000	
Cat wash 2 buffer	2000	
Elution buffer	2000	2000
Regeneration buffer	6000	
Cat ex waste		12000
<b>Endotoxin</b>	20.06	10.03
<b>Product</b>	9.04	8.77

Characteristics

Model: Chromaflow 1000  
Material of Construction: Stainless Steel 316L  
Finish: Electro-polished  
Bed Volume: 2,000L  
Column Diameter: 2.54m  
Bed Height: 0.5m  
Resin: Eshmuno S (EMD Millipore)  
Binding Capacity: > 60 mg antibody/ ml media  
Working Flow Velocity: 800 cm/hr

Operations Conditions

Temp: 25°C  
Pressure: 1 bar

Purchase Cost

Column: \$ 250,000  
Resin: \$ 15,000/10 Liters (need 200 of these)



**Cation Exchange Pool Tank (PFD 02/P-37)**

<u>Description and Function</u>	To hold and mix product from cation exchange chromatography with buffer solutions in preparation for anion exchange chromatography.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #02	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Total Volume:	5,000L
	Height:	3.0m
	Diameter:	1.5m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp :	25°C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$ 122,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Anion Exchange Chromatography Column (PFD 02/P-39)**

Description and Function To remove impurities and purify antigen binding fragments.

Vendor GE Healthcare

PFD Reference PFD #02

Operation Batch

	Input (kg/Batch)	Output (kg/Batch)
Cells	0	0
Media	0	0
<b>Water Streams:</b>		
Cat ex effluent	2000	
Cat wash 1	2000	
Cat wash 2	2000	
Elution buffer	2000	2000
Regeneration buffer	6000	
Anion ex waste		12000
<b>Endotoxin</b>	10.03	5.02
<b>Product</b>	8.77	8.50

Characteristics

Model:	Chromaflo 1000
Material of Construction:	Stainless Steel 316L
Finish:	Electro-polished
Bed Volume:	2,000L
Column Diameter:	2.54m
Bed Height:	0.5m
Resin:	Capto Q (GE Healthcare)
Binding Capacity:	> 100 mg antibody/mL media
Working Flow Velocity:	700 cm/hr

Operations Conditions

Temp:	25°C
Pressure:	1 bar

Purchase Cost

Column:	\$ 250,000
Resin:	\$ 109.00/60 liters (need 40 of these)

**Anion Exchange Pool Tank (PFD 02/P-41)**

<u>Description and Function</u>	To collect product from anion exchange column.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #02	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Total Volume:	25,000L
	Height:	5.0m
	Diameter:	2.5m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	25°C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$ 145,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Nanofiltration Unit (PFD 02/P-43)**

<u>Description and Function</u>	To remove viruses. It is a size based membrane separation in which the product flows through while viruses are captured within the pores.		
<u>Vendor</u>	Asahi Kasei Bioprocess		
<u>PFD Reference</u>	PFD #02		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0	0
	Media	0	0
	Water	2000	2000
	Endotoxin	5.02	2.51
	Product	8.50	8.42
<u>Characteristics</u>	Model:	Planova BioEx	
	MW Cutoff:	200 kD	
	Protein Throughput:	6.5 kg/m <sup>2</sup> per 3 hours	
	Filtration pressure:	294 kPa (42.6 psi)	
	Filter effective surface area:	1.8m <sup>2</sup>	
	Sterilization:	SIP (autoclave)	
<u>Operations Conditions</u>	Membrane:	Disposable	
	Temp:	25°C	
	Suggested Operating Pressure:	< 343 kPa	
	Operating pH:	2-9	
<u>Purchase Cost</u>	Filter Housing:	\$ 15,000	
	Membrane Filter:	\$ 2,000	

**Ultrafiltration (PFD 02/P-45)**

Description and Function To remove small viral and foreign particles. The desired product will stay in the retentate but smaller particles will be removed.

Vendor Pall Corporation

PFD Reference PFD #02

Operation Batch

		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
<u>Materials Handled</u>	Cells	0	0
	Media	0	0
	Water	2000	2000
	Endotoxin	2.51	1.25
	Product	8.42	8.08

<u>Characteristics</u>	Model:	Membrane Unit
	Disc Filters:	Omega polyethersulfone membrane
	MW Cutoff:	100 kD
	Filter effective surface area:	1.8m <sup>2</sup>
	Sterilization:	SIP (autoclave)
	Membrane Treatment:	Sterilized, washed, and reused

<u>Operations Conditions</u>	Temp:	0 - 40°C
	Operating Pressure:	1 bar

<u>Purchase Cost</u>	Filter Housing:	\$ 12,000
	Filter Membrane:	\$ 1,000

**Final Sterile Filtration (PFD 02/P-47)**

Description and Function .2 µm dead end filter to remove bacteria and other impurities from the serum free media prior to feeding the cultivators and production bioreactor. The sanitary stainless steel filter housings are designed for filtration applications. The filter capsules membranes are disposable ready-to-use filter or depth filter capsules.

Vendor Sartorius Biotech

PFD Reference PFD #02

Operation Batch

	<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
<u>Materials Handled</u>		
Cells	0	0
Media	0	0
Water	2000	2000
Endotoxin	1.25	0
Product	8.08	7.76

Characteristics

Model:	Sartopore 2 0.2 µm
Membrane Filter Material:	Polyethersulfone (PES)
Size:	30 inches
Filtration Area:	1.8m <sup>2</sup> (18ft <sup>2</sup> )
Max. Differential Pressure:	5 bar (75 psi) at 20 °C
Membrane Unit Sterilization:	SIP
Membrane Sterilization:	Disposable

Operations Conditions

Temp :	25°C
Pressure :	0.5 bar (7.5 psi)

Purchase Cost

Filter Housing:	\$ 2,000
Filter Membrane:	\$ 650/unit

## 9.2. Fluidized Bed Bioreactor Process

### Bag Bioreactor (PFD 03/P-02)

<u>Description and Function</u>	To prepare the inoculum for seed bioreactor. An electric rocker base, with integral temperature control, weight controllers for perfusion culture, aeration pump, pH probe, and various rocking speeds is used along with 200mL Cellbag bioreactor chambers. The bags are plastic pre-sterilized, single-use chambers for non-invasive mixing of fluids using the WAVE <sup>TM</sup> rocker.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #03		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0.00524	0.0262
	Media	0.002	0
	Water	0.098	0.098
	Endotoxin	0	0
	Product	0	0
<u>Characteristics</u>	Model :	GE Healthcare WAVE <sup>TM</sup> Base 2/10EH	
	Construction:	High quality stainless steel and aluminum	
	Bag Bioreactor :	Cellbag 200mL Bioreactor Chamber	
	Sterilization:	Plastic Disposable Bags	
		SIP for rocker	
<u>Operations Conditions</u>	Temp :	37° C	
	Pressure :	1 bar	
	pH :	7.0 – 7.2	
	DO :	50%	
	Duration:	3.5 days	
<u>Purchase Cost</u>	Rocker and Control Unit:	\$16,480	
	200 mL Cellbag Bioreactor Chamber:	\$202.00 per bag (need 30)	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500	

**Media Prep (PFD 03/P-03)**

Description and Function      Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor      Sharpsville Container

PFD Reference      PFD #03

Operation      Batch

	Input	Quantity (L)	Output	Quantity (L)
<u>Materials Handled</u>	Dry Powder Media	0.2	Media Solution	98.00
	Water	97.8		

Characteristics

Material of Construction:	Stainless Steel 316L
Volume:	150L
Height:	0.9m
Diameter:	0.4m
Sterilization:	SIP/CIP

Operations Conditions

Temp:	25°C
Pressure:	1 bar

Purchase Cost

Storage Tank:	\$ 110,000
PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500



**Seed Bioreactor (PFD 03/P-07)**

<u>Description and Function</u>	Bioreactor used for continued cell growth and production of monoclonal antibodies. The WAVE™ Bioreactor 500/1000 is a self-contained system with integral temperature control, aeration pump, and rocking controller for use with working culture volumes between 50L and 500L. The 70L bags are plastic pre-sterilized, single-use chambers for non-invasive mixing of fluids using the WAVE™ bioreactor.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #03		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0.26	1.75
	Media	2	0
	Water	98	98
	Endotoxin	0	0.47
	Product	0	0.02
<u>Characteristics</u>	Model:	WAVE™ Bioreactor 500/1000	
	Construction:	Stainless Steel	
	Dimensions:	201x124x160 cm	
	Bag Bioreactor:	Cellbag 70L Bioreactor Chamber	
	Sterilization:	Plastic Disposable Bags	
<u>Operations Conditions</u>	Temp:	37° C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	50%	
	Duration:	5 days	
<u>Purchase Cost</u>	WAVE™ Bioreactor system:	\$ 404,500 (3x)	
	70L Cellbag Bioreactor Chamber:	\$ 1,000 per bag	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500	

**Media Prep (PFD 03/P-09)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #03

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Media	18.00	Media	900.0
	Powder		Solution	
	Water	882.0		

<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	1500L
	Height:	2.3m
	Diameter:	0.9m
	Sterilization:	SIP/CIP

<u>Operations Conditions</u>	Temp :	25°C
	Pressure :	1 bar

<u>Purchase Cost</u>	Storage Tank:	\$ 120,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Seed Bioreactor (PFD 03/P-13)**

<u>Description and Function</u>	Bioreactor used for continued cell growth and production of monoclonal antibodies. The XDR-2000 Single-Use Bioreactor offers a smooth transfer from development to manufacturing scale. It has robust industrial automation with an aeration pump. It provides exacting temperature control for use with working culture volumes up to 2000L. The 2000L bags are plastic pre-sterilized, single-use chambers for non-invasive mixing of fluids using the XDR bioreactor.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #03		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	5.24	38.25
	Media	20	0
	Water	980	980
	Endotoxin	1.40	10.20
	Product	0.05	0.46
<u>Characteristics</u>	Model:	XDR-2000 Single-Use Bioreactor	
	Construction:	Jacketed stainless steel vessel	
	Bag Bioreactor:	Cellbag 2000L Bioreactor Chamber	
	Sterilization:	Plastic Disposable Bags	
<u>Operations Conditions</u>	Temp:	37°C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	50%	
	Duration:	5.5 days	
<u>Purchase Cost</u>	WAVE™ Bioreactor system:	\$ 600,000	
	125L Cellbag Bioreactor Chamber:	\$ 1,800 per bag	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500	

**Media Prep (PFD 03/P-15)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #03

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Media	8.6E+05	Media	4.29E+06
	Powder		Solution	
	Water	4.21E+06		

<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	10,000L
	Height:	3.5m
	Diameter:	2.0m
	Sterilization:	SIP/CIP

<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	1 bar

<u>Purchase Cost</u>	Storage Tank:	\$ 130,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Media Prep (PFD 03/P-19)**

Description and Function Tank used to store and mix powdered serum free media with water for injection prior to being pumped through a sterile filter.

Vendor Sharpsville Container

PFD Reference PFD #03

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Media	8.6E+05	Media	4.29E+06
	Powder		Solution	
	Water	4.21E+06		

<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished finish
	Volume:	10,000L
	Height:	3.5m
	Diameter:	2.0m
	Sterilization:	SIP/CIP

<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	1 bar

<u>Purchase Cost</u>	Storage Tank:	\$ 130,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Media Storage (PFD 03/P-23)**

Description and Function To store media before entering the production fluidized bioreactor.

Vendor Sharpsville Container

PFD Reference PFD #03

Operation Batch

	<b>Input</b>	<b>Quantity (L)</b>	<b>Output</b>	<b>Quantity (L)</b>
<u>Materials Handled</u>	Dry Media	1.72E+05	Media	8.60E+06
	Powder		Solution	
	Water	8.42E+06		

<u>Characteristics</u>	Model:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	50,000L
	Height:	6.0m
	Diameter:	3.25m
	Sterilization:	SIP/CIP

<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	1 bar

<u>Purchase Cost</u>	Storage Tank:	\$ 165,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Production Bioreactor (PFD 03/P-25)**

<u>Description and Function</u>	Fluidized Bioreactor with internal circulation used for continued cell growth and production of monoclonal antibodies. The final bioreactor serves to maximize product formation.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #03		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	38.25	2672.4
	Media	1.72E+05	1.17E+05
	Water	8.42E+06	5.90E+06
	Endotoxin	10.20	272.58
	Product	0.46	65.75
<u>Characteristics</u>	Model:	25,000L Cytopilot™ Production Bioreactor	
	Material:	Stainless Steel 316L	
	Finish:	Electro-polished	
	Sterilization:	SIP/CIP	
<u>Operations Conditions</u>	Temp:	37° C	
	Pressure:	1 bar	
	pH:	7.0 – 7.2	
	DO:	40%	
	Duration:	13 days	
<u>Purchase Cost</u>	Bioreactor:	\$1,500,000	
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$2,500/control = \$12,500	

**Sterile Filtration (PFD 03/P-05, P-11, P-17, P-21)**

<u>Description and Function</u>	.2 µm dead end filter to remove bacteria and other impurities from the serum free media prior to feeding the cultivators and production bioreactor. The sanitary stainless steel filter housings are designed for filtration applications. The filter capsules membranes are disposable ready-to-use filter or depth filter capsules.	
<u>Vendor</u>	Sartorius Biotech	
<u>PFD Reference</u>	PFD #03	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	Sartopore 2 0.2 µm
	Membrane Filter Material:	Polyethersulfone (PES)
	Size:	30"
	Filtration Area:	1.8m <sup>2</sup> (18ft <sup>2</sup> )
	Max. Differential Pressure:	5 bar (75 psi) at 20 °C
	Membrane Unit Sterilization:	SIP
	Membrane Sterilization:	Disposable
<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	0.5 bar (7.5 psi)
<u>Purchase Cost</u>	Filter Housing:	\$ 2,000
	Filter Membrane:	\$ 650/unit



**Pump (PFD 03 & 04/ P-04, P-06, P-08, P-10, P-12, P-14, P-16, P-18, P-20, P-22, P-24, P-26, P-28, P-30, P-32, P-34, P-36, P-38, P-40, P-42, P-44, P-46, P-48, P-50, P-51)**

<u>Description and Function</u>	Pump to transfer fluid. High-flow hygienic pumps that are designed for low-shear sanitary pumping. They are ideal for viscous or shear sensitive products.	
<u>Vendor</u>	Watson-Marlow	
<u>PFD Reference</u>	PFD #03 and #04	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Model:	825 Peristaltic Pump
	Material Construction:	304 Stainless Steel
	Flow Rate:	33.3 L/min
	Max Pressure :	3.5 bar
	Tubing:	Bioprene, 25mm
<u>Operations Conditions</u>	Temp:	25°C
	Pressure Change:	50 psi
	Power:	1.5 – 5.0 hp
<u>Purchase Cost</u>	\$13,000	

**Storage Tank (PFD 04/P-27)**

<u>Description and Function</u>	To store output of production bioreactor before tangential flow filtration.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #04	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	50,000L
	Height:	6.0m
	Diameter:	3.25m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	25°C
	Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$165,000 (x58)
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$2,500/control = \$12,500 (x58)

**Tangential Flow Filtration (PFD 04/P-29)**

<u>Description and Function</u>	To remove cells, viruses, and bacteria from the stream containing the monoclonal antibody product. It a process by which the product or feed is directed tangentially along the surface of the membrane.		
<u>Vendor</u>	Pall Corporation		
<u>PFD Reference</u>	PFD #04		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	2672.4	0
	Media	1.17E+05	0
	Water	5.90E+06	5.84E+06
	Endotoxin	272.58	250.78
	Product	65.75	64.43
<u>Characteristics</u>	Model:	Cadence Single Pass TFF System	
	Material:	Stainless Steel 316L	
	Finished:	Electro-polished	
	Sterilization:	SIP/CIP	
<u>Operations Conditions</u>	Temp:	4-40°C	
	Flow Range:	6-1,000 L/h	
	Pressure Range:	4-6 bar	
	pH Range:	2-13	
<u>Purchase Cost</u>	\$500,000 (x50)		

**TFF Pool Tank (PFD 04/P-31)**

<u>Description and Function</u>	To store sterilized supernatant from the tangential flow filtration process.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #04	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	50,000 L
	Height:	6 m
	Diameter:	3.25 m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	25 ° C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$145,000 (x58)
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500 (x58)

**Protein A Chromatography Column (PFD 04/P-33)**

<u>Description and Function</u>	To purify the antibody Fab fragments. It acts as the first main isolation step for product and isolates the monoclonal antibody via affinity chromatography. Therefore, the column is useful in eliminating host cell DNA, media components, and endogenous viral particles.		
<u>Vendor</u>	GE Healthcare		
<u>PFD Reference</u>	PFD #04		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0	0
	Media	0	0
	<b>Water Streams:</b>		
	Centrifuge effluent	5.84E+06	
	Equilibration buffer	2000	
	Wash buffer	2000	
	Elution buffer	4000	4000
	Regeneration buffer	6000	
	Protein A waste		5.85E+06
	<b>Endotoxin</b>	250.78	130.40
	<b>Product</b>	64.43	63.14
	<u>Characteristics</u>	Model:	Chromaflow 2000/ 100-300
Column Diameter:		2.54m	
Bed Height:		0.4m	
Material of Construction:		Stainless Steel 316L	
Bed Volume:		2000L	
Resin		n-Protein A Sepharose 4	
Binding Capacity:		50 mg antibody / ml media	
Working Flow Velocity:		40 cm/min	
<u>Operations Conditions</u>	Temperature Stability:	4-40 °C	
	Temp:	4 °C	
<u>Purchase Cost</u>	Pressure:	1.01 bar	
	Column:	\$ 200,000	
	Resin:	\$ 8,000/ liters (need 2,000 L)	

**Protein A Pool Tank (PFD 04/P-35)**

<u>Description and Function</u>	To collect product from anion exchange column.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #04	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Total Volume:	5,000.0 L
	Height:	3.0 m
	Diameter:	1.5 m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	25 ° C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$ 122,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Cation Exchange Chromatography Column (PFD 04/P-37)**

Description and Function To purify and isolate antigen binding fragments from impurities.

Vendor GE Healthcare

PFD Reference PFD #04

Operation Batch

	Input (kg/Batch)	Output (kg/Batch)
<b>Cells</b>	0	
<b>Media</b>	0	
<b>Water Streams:</b>		
Prot A effluent	2000	
Cat wash 1 buffer	2000	
Cat wash 2 buffer	2000	
Elution buffer	2000	2000
Regeneration buffer	6000	
Cat ex waste		12000
<b>Endotoxin</b>	130.40	50.16
<b>Product</b>	63.14	61.25

Characteristics

Model:	Chromaflow 1000
Material of Construction:	Stainless Steel 316L
Finish:	Electro-polished
Bed Volume:	2,000.0 L
Column Diameter:	2.54 m
Bed Height:	0.5 m
Resin:	Eshmuno S (EMD Millipore)
Binding Capacity:	> 60 mg antibody/ ml media
Working Flow Velocity:	800 cm/hr

Operations Conditions

Temp :	25 ° C
Pressure:	1 bar

Purchase Cost

Column:	\$ 250,000
Resin:	\$ 15,000/10 Liters (need 200 of these)

**Cation Exchange Pool Tank (PFD 04/P-39)**

<u>Description and Function</u>	To hold and mix product from cation exchange chromatography with buffer solutions in preparation for anion exchange chromatography.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #04	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Volume:	5,000.0 L
	Height:	3.0 m
	Diameter:	1.5 m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	25 ° C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$ 122,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500



**Anion Exchange Chromatography Column (PFD 04/P-41)**

Description and Function To remove impurities and purify antigen binding fragments

Vendor GE Healthcare

PFD Reference PFD #04

Operation Batch

	Input (kg/Batch)	Output (kg/Batch)
<b>Cells</b>	0	
<b>Media</b>	0	
<b>Water Streams:</b>		
Cat ex effluent	2000	
Anion wash 1 buffer	2000	
Anion wash 2 buffer	2000	
Elution buffer	2000	2000
Regeneration buffer	6000	
Anion ex waste		12000
<b>Endotoxin</b>	50.16	30.09
<b>Product</b>	61.25	59.41

Characteristics

Model: Chromaflow 1000  
 Material of Construction: Stainless Steel 316L  
 Finish: Electro-polished  
 Bed Volume: 2,000.0 L  
 Column Diameter: 2.54 m  
 Bed Height: 0.5 m  
 Resin : Capto Q (GE Healthcare)  
 Binding Capacity: > 100 mg antibody/ ml media  
 Working Flow Velocity: 700 cm/hr

Operations Conditions

Temp: 25 °C  
 Pressure: 1 bar

Purchase Cost

Column: \$ 250,000  
 Resin: \$ 109.00/60 Liters (need 40 of these)

**Anion Exchange Pool Tank (PFD 04/P-43)**

<u>Description and Function</u>	To collect product from anion exchange column.	
<u>Vendor</u>	Sharpsville Container	
<u>PFD Reference</u>	PFD #04	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finish:	Electro-polished
	Total Volume:	25,000.0 L
	Height:	5.0 m
	Diameter:	2.5 m
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	25 ° C
	Operating Pressure:	1 bar
<u>Purchase Cost</u>	Storage Tank:	\$ 145,000
	PI control for pH, Temp, DO, Flow Rate, Volume, and Pressure:	\$ 2,500/control = \$12,500

**Viral Nanofiltration Unit (PFD 04/P-45)**

<u>Description and Function</u>	To remove viruses. It is a size based membrane separation in which the product flows through and the viruses are captured within the pores.		
<u>Vendor</u>	Asahi Kasei Bioprocess		
<u>PFD Reference</u>	PFD #04		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0	0
	Media	0	0
	Water	2000	2000
	Endotoxin	30.09	10.03
	Product	59.41	58.82
<u>Characteristics</u>	Model:	Planova BioEx (EX1-0000)	
	MW Cutoff:	200 kD	
	Protein Throughput:	6.5 kg/m <sup>2</sup> per 3 hours	
	Filtration pressure:	294 kPa (42.6 psi)	
	Filter effective surface area:	1.8 m <sup>2</sup>	
	Sterilization:	SIP (autoclave)	
	Membrane:	Disposable	
<u>Operations Conditions</u>	Temp:	25 ° C	
	Suggested Operating Pressure:	<343 kPa	
	Operating pH:	2-9	
<u>Purchase Cost</u>	Filter Housing:	\$ 15,000 (x6)	
	Membrane Filter:	\$ 2,000	

**Ultrafiltration (PFD 04/P-47)**

Description and Function To remove small viral and foreign particles. The desired product will stay in the retentate but smaller particles will be removed.

Vendor Pall Corporation

PFD Reference PFD #04

Operation Batch

	Input (kg/Batch)	Output (kg/Batch)
<u>Materials Handled</u> Cells	0	0
Media	0	0
Water	2000	2000
Endotoxin	10.03	3.34
Product	58.82	56.47

<u>Characteristics</u> Model:	Membrane Unit
Disc Filters:	Omega polyethersulfone membrane
MW Cutoff:	100 kD
Filter effective surface area:	1.8 m <sup>2</sup>
Sterilization:	SIP (autoclave)
Membrane Treatment:	Sterilized, washed, and reused

<u>Operations Conditions</u> Temp:	0 - 40°C
Operating Pressure:	1 bar

<u>Purchase Cost</u> Filter Housing:	\$ 12,000 (x6)
Filter Membrane:	\$ 1,000

**Sterile Filtration (PFD 04/P-49)**

<u>Description and Function</u>	.2 µm dead end filter to remove bacteria and other impurities from the serum free media prior to feeding the cultivators and production bioreactor. The sanitary stainless steel filter housings are designed for filtration applications. The filter capsules membranes are disposable ready-to-use filter or depth filter capsules.		
<u>Vendor</u>	Sartorius Biotech		
<u>PFD Reference</u>	PFD #04		
<u>Operation</u>	Batch		
<u>Materials Handled</u>		<b>Input (kg/Batch)</b>	<b>Output (kg/Batch)</b>
	Cells	0	0
	Media	0	0
	Water	2000	2 00
	Endotoxin	3.34	0
	Product	56.47	54.21
<u>Characteristics</u>	Model:	Sartopore 2 0.2 µm	
	Membrane Filter Material:	Polyethersulfone (PES)	
	Size:	30"	
	Filtration Area:	1.8 m <sup>2</sup> (18 ft <sup>2</sup> )	
	Max. Differential Pressure:	5 bar (75 psi) at 20 °C	
	Membrane Unit Sterilization:	SIP	
<u>Operations Conditions</u>	Membrane Sterilization:	Disposable	
	Temp:	25 °C	
<u>Purchase Cost</u>	Pressure:	0.5 bar (7.5 psi)	
	Filter Housing:	\$ 2,000	
	Filter Membrane:	\$ 650/unit	

**High Temperature Inactivation (PFD 05/P-52)**

<u>Description and Function</u>	An industrial size autoclave sterilizer that serves to kill the cells attached to the microcarriers in order to allow for safe disposal.	
<u>Vendor</u>	WSF Industries Inc.	
<u>PFD Reference</u>	PFD #05	
<u>Operation</u>	Batch	
<u>Characteristics</u>	Material of Construction:	Stainless Steel 316L
	Finished:	Electro-polished
	Volume:	5,000L
	Sterilization:	SIP/CIP
<u>Operations Conditions</u>	Temp:	250°C
	Pressure:	20-30 psi
<u>Purchase Cost</u>	\$ 500,000	

## **10. Overall Process Cost for Stirred Tank Bioreactor Process**

### **10.1. Utility Costs**

The major utility needs of the plant consists of electricity and process water. Electricity is the most expensive and costs approximately \$38,250 per batch. This calculation is based off a \$0.085 per kWh rate obtained from a Philadelphia energy provider. 450,000 kWh of energy are used per batch for the tank process. These energy requirements are due to operation of process machinery, the production bioreactor, the pumps, the WFI still, the clean steam generators, and other various units.

Process water is the other major utility that will be used to produce monoclonal antibodies. The plant will require about 108,000 kg/batch, which is approximately 2,052,000kg per year. This water will be used to generate WFI and steam for buffers and SIP in addition to the water needed for the powder media. At a rate of \$0.00473/kg, a rate obtained from a Philadelphia water company, the costs of process water will be about \$9,700 per year.

### **10.2. Material Resource Costs**

The costs of media and buffers can be found in this section. Ex-Cell<sup>®</sup> ACF CHO dry powder media can be purchased from Sigma-Aldrich<sup>™</sup> for \$35.20 per kilogram. Approximately 10,000kg of dry media are needed for each batch when using the tank process. All of the buffers for the Protein A, Cation Exchange, and Anion Exchange columns can be purchased from GE Healthcare at a price of \$1.24 per kilogram. Approximately 460,000kg of buffers are need per batch for the tank process.

### 10.3. Major Unit Operation Costs

Innoculum Prep - Bioreaction					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-02	Bioreactor Rocker	1		GE Healthcare	\$16,480.00
	Bag	20	200mL	GE Healthcare	\$202.00
P-03	Mixing Tank	1	150L	Sharpville Container	\$110.00
P-04	Pump	1		Watson Marlow	\$13,000.00
P-05	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-06	Pump	1		Watson Marlow	\$13,000.00
P-07	WAVE™ Bioreactor System	1		GE Healthcare	\$404,500.00
	Bag	1	200L	GE Healthcare	\$1,200.00
P-08	Pump	1		Watson Marlow	\$13,000.00
P-09	Mixing Tank	1	1,500L	Sharpville Container	\$1200.00
P-10	Pump	1		Watson Marlow	\$13,000.00
P-11	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-12	Pump	1		Watson Marlow	\$13,000.00
P-13	Bioreactor System	1		GE Healthcare	\$600,000.00
	Bag	1	2,000L	GE Healthcare	\$1,800.00
P-14	Pump	1		Watson Marlow	\$13,000.00
P-15	Mixing Tank	1	10,000L	Sharpville Container	\$130,000.00
P-16	Pump	1		Watson Marlow	\$13,000.00
P-17	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-18	Pump	1		Watson Marlow	\$13,000.00
P-19	Mixing Tank	1	10,000L	Sharpville Container	\$130,000.00
P-20	Pump	1		Watson Marlow	\$13,000.00
P-21	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-22	Pump	1		Watson Marlow	\$13,000.00
P-23	Production Bioreactor	1	25,000L	Techniserv, Inc	\$900,000.00



Primary Recovery					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-24	Pump	1		Watson Marlow	\$13,000.00
P-25	Storage Tank	1	25,000L	Sharpsville Container	\$145,000.00
P-26	Pump	1		Watson Marlow	\$13,000.00
P-27	Centrifuge	1	10,000 L/hr	Alfa-Laval	\$480,000.00
P-28	Pump	1		Watson Marlow	\$13,000.00
P-29	Centrifugation Pool Tank	1	25,000L	Sharpsville Container	\$145,000.00

Protein A Chromatography					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-30	Pump	1		Watson Marlow	\$13,000.00
P-31	Protein A Column	1	2,000L	GE Healthcare	\$200,000.00
	n-Protein A Sepharose 4 Resin	2,000	1L	GE Healthcare	\$8,000.00
P-32	Pump	1		Watson Marlow	\$13,000.00
P-33	Protein A Pool Tank	1	5,000L	Sharpsville Container	\$122,000.00

Cation Exchange Chromatography					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-34	Pump	1		Watson Marlow	\$13,000.00
P-35	Cation Exchange Column	1	2,000L	GE Healthcare	\$250,000.00
	Eshmuno S Resin	200	10L	EMD Millipore	\$15,000.00
P-36	Pump	1		Watson Marlow	\$13,000.00
P-37	Cation Exchange Pool Tank	1	5,000L	Sharpsville Container	\$122,000.00

Anion Exchange Chromatography					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-38	Pump	1		Watson Marlow	\$13,000.00
P-39	Anion Exchange Column	1	2,000L	GE Healthcare	\$250,000.00
	Capto Q Resin	40	60L	GE Healthcare	\$109,000
P-40	Pump	1		Watson Marlow	\$13,000.00
P-41	Anion Exchange Pool Tank	1	25,000L	Sharpsville Container	\$145,000.00

Viral Nanofiltration					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-42	Pump	1		Watson Marlow	\$13,000.00
P-43	Nanofiltration Housing Unit	6		Asahi Kasei Bioprocess	\$15,000.00
	Filters	1	1.8m <sup>2</sup>	Asahi Kasei Bioprocess	\$2,000.00

Final Filtration					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-44	Pump	1		Watson Marlow	\$13,000.00
P-45	Ultrafiltration Unit	6	1.8m <sup>2</sup>	Pall Corporation	\$12,000.00
	Filters	1		Pall Corporation	\$1,000.00
P-46	Pump	1		Watson Marlow	\$13,000.00
P-47	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-48	Pump	1		Watson Marlow	\$13,000.00

#### 10.4. Additional Equipment Costs

Additional Equipment			
Type	Units	Vendor	Purchase Cost (\$/Unit)
Incubators	2	Thermo Scientific	\$15,000.00
CIP Skids	3	Sani-matic	\$100,000.00
Buffer Transfer Bags	80	HyClone – (Thermo Scientific)	80,000/year
Portable Pump	3	Watson Marlow	\$13,000.00
Biowaste and Neutralization Tanks	5	Sharpsville Container	\$145,000.00
Final Packaging	1	Quality BioResources, Inc.	\$280,000.00

Additional Equipment Included in Annual Expenses		
Type	Units	Vendor
Biosafety Cabinet	3	Thermo Scientific
Air Generator (HVAC Equipment)	1	
Clean Steam Generator	1	
Filter Integrity Tester	1	Millipore
Tube Fuser and Sealer	1	Sartorius Stedim Biotech
Water Treatment Package	1	
WFI Still	1	Paul Mueller Co
Biowaste Inactivation System	1	
Waste Neutralization System	1	
Laboratory Information Management System	1	
Refrigeration	2	Thermo Scientific

## 11. Overall Process Cost for Fluidized Bioreactor Process

### 11.1. Utility Costs

The major utility needs of the plant consists of electricity and process water.

Electricity is the most expensive and costs approximately \$59,500 per batch. This calculation is based off a \$0.085 per kWh rate obtained from a Philadelphia energy provider.

700,000kWh of energy are used per batch for the stirred tank process. These electricity requirements are higher than the stirred tank process due to the increased number of pumps and the increased amount of water. Again, electricity is required for operation of process machinery, the production bioreactor, the pumps, the WFI still, the clean steam generators, and other various units.

Process water is the other major utility that will be used to produce monoclonal antibodies. The plant will require about 8,500,000kg/batch, which is approximately 100 million kg per year. This large amount of increased water is due to the increased amount of media for the fluidized bed process. Again, this water will be used to generate WFI and steam for buffers and SIP in addition to the water needed to mix with the media. At a rate of \$0.00473/kg, a rate obtained from a Philadelphia water company, the costs of process water will be about \$40,205 per batch.

### 11.2. Material Resource Costs

The costs of media, buffers, and microcarriers can be found in this section. Ex-Cell<sup>®</sup> ACF CHO dry powder media can be purchased from Sigma-Aldrich<sup>™</sup> for \$35.20 per kilogram. Approximately 2 million kg of dry media are needed for each batch when using the fluidized bed system. All of the buffers for the Protein A, Cation Exchange, and Anion Exchange columns can be purchased from GE Healthcare at a price of \$1.24 per kilogram. Approximately 400,000kg of buffers are need per batch for the fluidized bed process. Lastly, the Cytoline<sup>™</sup> 1 microcarriers can be purchased from GE Healthcare at a cost of \$4,488 per kilogram. Approximately 12,000kg of microcarriers are needed for the production bioreactor for each batch.

### 11.3. Major Unit Operation Costs

Innoculum Prep - Bioreaction					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-02	Bioreactor Rocker	1		GE Healthcare	\$16,480.00
	Bag	30	200mL	GE Healthcare	\$202.00
P-03	Mixing Tank	1	150L	Sharpsville Container	\$110.00
P-04	Pump	1		Watson Marlow	\$13,000.00
P-05	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-06	Pump	1		Watson Marlow	\$13,000.00
P-07	WAVE™ Bioreactor System	3		GE Healthcare	\$404,500.00
	Bag	1	70L	GE Healthcare	\$1,000.00
P-08	Pump	1		Watson Marlow	\$13,000.00
P-09	Mixing Tank	1	1,500L	Sharpsville Container	\$120.00
P-10	Pump	1		Watson Marlow	\$13,000.00
P-11	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-12	Pump	1		Watson Marlow	\$13,000.00
P-13	Bioreactor System	1		GE Healthcare	\$600,000.00
	Bag	1	2,000L	GE Healthcare	\$1,800.00
P-14	Pump	1		Watson Marlow	\$13,000.00
P-15	Mixing Tank	1	10,000L	Sharpsville Container	\$130,000.00
P-16	Pump	1		Watson Marlow	\$13,000.00
P-17	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-18	Pump	1		Watson Marlow	\$13,000.00
P-19	Mixing Tank	1	10,000L	Sharpsville Container	\$130,000.00
P-20	Pump	1		Watson Marlow	\$13,000.00
P-21	Sterile Filter	1	1.8 m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-22	Pump	1		Watson Marlow	\$13,000.00
P-23	Media Storage Tank	1	50,000L	Sharpsville Container	\$165,000.00
P-24	Pump	1		Watson Marlow	\$13,000.00
P-25	Production Bioreactor	1	25,000L	GE Healthcare	\$1,500,000.00

Primary Recovery					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-26	Pump	1		Watson Marlow	\$13,000.00
P-27	Storage Tank	58	50,000L	Sharpsville Container	\$165,000.00
P-28	Pump	58		Watson Marlow	\$13,000.00
P-29	Tangential Flow Filtration	50		Pall Corporation	\$500,000.00
P-30	Pump	50		Watson Marlow	\$13,000.00
P-31	TFF Pool Tank	58	50,000L	Sharpsville Container	\$165,000.00

Protein A Chromatography					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-32	Pump	58		Watson Marlow	\$13,000.00
P-33	Protein A Column	1	2,000L	GE Healthcare	\$200,000.00
	n-Protein A Sepharose 4 Resin	5	1L	GE Healthcare	\$8,000.00
P-34	Pump	1		Watson Marlow	\$13,000.00
P-35	Protein A Pool Tank	1	5,000L	Sharpsville Container	\$122,000.00

Cation Exchange Chromatography					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-36	Pump	1		Watson Marlow	\$13,000.00
P-37	Cation Exchange Column	1	2,000L	GE Healthcare	\$250,000.00
	Eshmuno S Resin	1	10L	EMD Millipore	\$15,000.00
P-38	Pump	1		Watson Marlow	\$13,000.00
P-39	Cation Exchange Pool Tank	1	5,000L	Sharpsville Container	\$122,000.00

Anion Exchange Chromatography					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-40	Pump	1		Watson Marlow	\$13,000.00
P-41	Anion Exchange Column	1	2,000L	GE Healthcare	\$250,000.00
	Capto Q Resin	40	60L	GE Healthcare	\$109,000
P-42	Pump	1		Watson Marlow	\$13,000.00
P-43	Anion Exchange Pool Tank	1	25,000L	Sharpsville Container	\$145,000.00

Viral Nanofiltration					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-44	Pump	1		Watson Marlow	\$13,000.00
P-45	Nanofiltration Housing Unit	6		Asahi Kasei Bioprocess	\$15,000.00
	Filters	1	1.8m <sup>2</sup>	Asahi Kasei Bioprocess	\$2,000.00

Final Filtration					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-46	Pump	1		Watson Marlow	\$13,000.00
P-47	Ultrafiltration Unit	6	1.8 m <sup>2</sup>	Pall Corporation	\$12,000.00
	Filters	1		Pall Corporation	\$1,000.00
P-48	Pump	1		Watson Marlow	\$13,000.00
P-49	Sterile Filter	1	1.8m <sup>2</sup>	Sartorius Biotech	\$2,000.00
	Filter Membrane	1		Sartorius Biotech	\$650.00
P-50	Pump	1		Watson Marlow	\$13,000.00

Microcarrier Handling					
Number	Type	Units	Size	Vendor	Purchase Cost (\$/unit)
P-51	Pump	1		Watson Marlow	\$13,000.00
P-52	Autoclave Sterilizer	1	5,000L	WSF Industries Inc	\$500,000.00

#### 11.4. Additional Equipment Costs

Additional Equipment			
Type	Units	Vendor	Purchase Cost (\$/Unit)
Incubators	2	Thermo Scientific	\$15,000.00
CIP Skids	3	Sani-matic	\$100,000.00
Buffer Transfer Bags	80	HyClone (Thermo Scientific)	80,000/year
Portable Pump	3	Watson Marlow	\$13,000.00
Biowaste and Neutralization Tanks	5	Sharpsville Container	\$145,000.00
Final Packaging	1	Quality BioResources, Inc.	\$280,000.00

Additional Equipment Included in Annual Expenses		
Type	Units	Vendor
Biosafety Cabinet	3	Thermo Scientific
Air Generator (HVAC Equipment)	1	
Clean Steam Generator	1	
Filter Integrity Tester	1	Millipore
Tube Fuser and Sealer	1	Sartorius Stedim Biotech
Water Treatment Package	1	
WFI Still	1	Paul Mueller Co
Biowaste Inactivation System	1	
Waste Neutralization System		
Laboratory Information Management System	1	
Refrigeration	2	Thermo Scientific



## 12. Scheduling

Scheduling is an important aspect to consider when building biopharmaceutical plants. Each process will be operated for about 330 days per year, allowing for about 35 days for maintenance and any contingency time needed in case of plant shutdown during operation. A Gantt chart can be used to easily visualize the occupation times of each piece of equipment in a given process to help identify any bottlenecks or inefficient design strategies. A two-batch Gantt chart for each process is included below. Only major units are shown and operation time includes transfer in and out times, loading times, and CIP and SIP times.

### 12.1. Gantt Chart

#### 12.1.1. Stirred Tank Bioreactor Process

The Gantt chart for the stirred tank bioreactor process is shown in Figure 12.1. Though this chart only shows two batches (the first batch in green and the second in purple), 16 total batches can be run every year in about 330 days. The first batch will require about 33 days and each incremental batch will require an additional 16 days.

#### 12.1.2. Fluidized Bioreactor Process

Figure 12.2 shows the Gantt chart for the fluidized bioreactor process. For this process, 24 batches can be run every year in about 330 days. The first batch requires about 32 days and each incremental batch will require an additional 13 days.

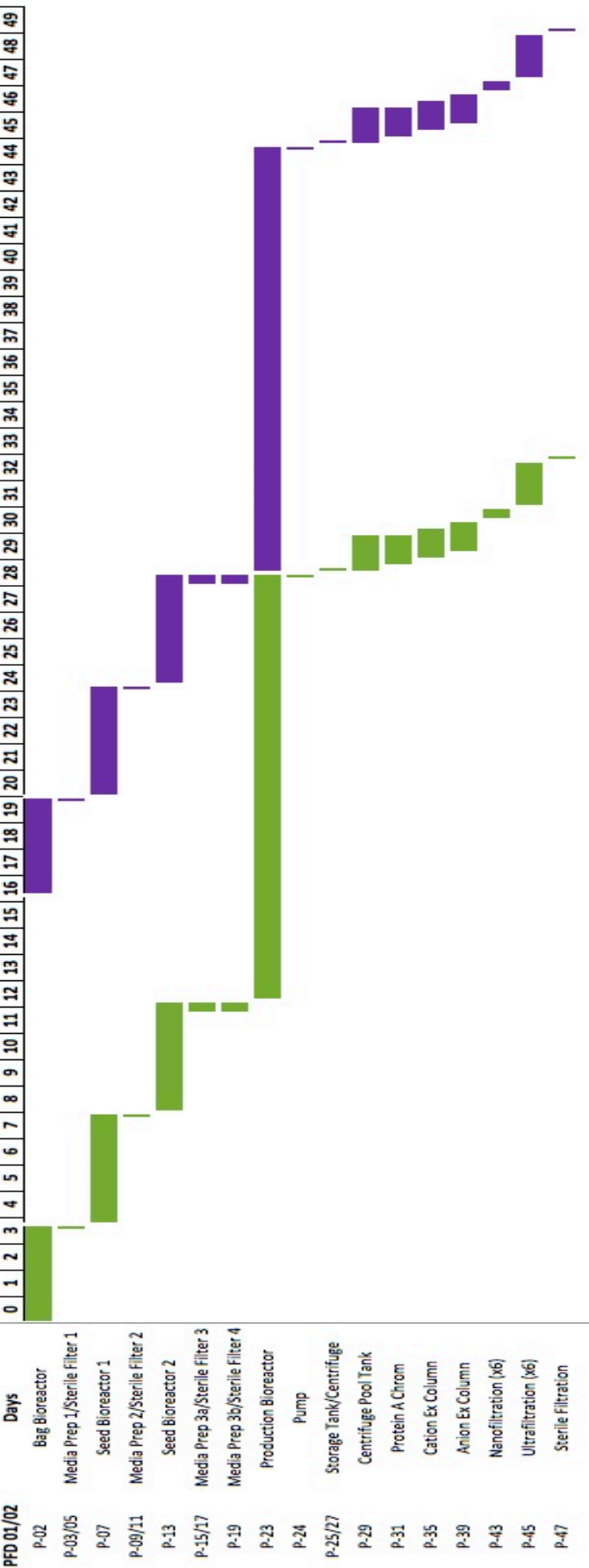


Figure 12.1

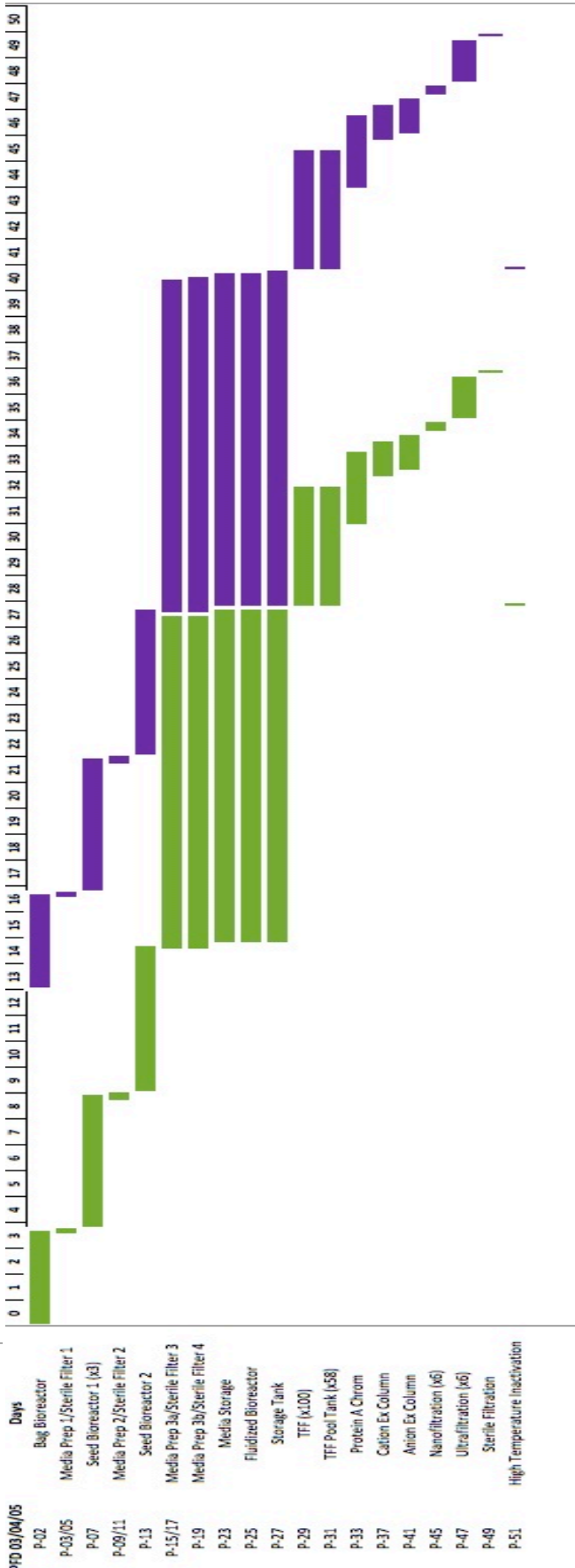


Figure 12.2

## 13. Economic Analysis

The fluidized bioreactor process offers the advantage of a lower batch time, fewer required batches per year, and a significant increase in product formation. However, it also requires investment in additional equipment, increases the sizes of various upstream and downstream units such as storage tanks or tangential filtration units. A profitability analysis can more accurately compare the fluidized bioreactor process to the traditional tank process to help determine which is the most fiscally conservative option.

### 13.1. Market Analysis

In the European Union (EU), Rituxan<sup>®</sup>'s patent will expire in 2014 and in 2018 in the United States (US). This creates an incredible opportunity for generic competition. With various blockbuster drugs also approaching a “patent cliff,” the biotechnology market, particularly the generic monoclonal antibody market, will be booming. In 2013, 560kg and 370kg of Rituxan<sup>®</sup> was sold in the US and EU respectively. Table 13.1 below shows the projected fractional market share of generic rituximab. It is projected that in the first year of a patent expiration, a generic is able to capture 50% of the market and that the market share increases every year until reaching a steady rate of 70%. Assuming that by 2021, 70% of the international and domestic market will be controlled by generics, the facility will be designed to produce 650kg of rituximab after accounting for downstream production losses.

**Table 13.1:** Expected market share projections for the European Union and United States markets. The expected market share will determine the amount of generic rituximab sold to customers.

Year	Fractional EU Market Share	Fractional US Market Share	Total Kg Sold by Facility
2015	0.50		279
2016	0.60		335
2017	0.70		391
2018	0.70		391
2019	0.70	0.50	576
2020	0.70	0.60	613
2021	0.70	0.70	650

13.2. Profitability Analysis

For the profitability analysis, it is assumed that the plant’s effective tax rate will be 22%, which reflects the sector average tax rate, and that the cost of capital for the plant will be 18%. A higher cost of capital was assumed due to the riskiness of the project and is also a reflection of the sector average.

The economic analysis shows that the stirred tank process has a net present value (at 18% and a life of 12 years) of about \$3.8 billion. It has a return on investment (ROI) of 249% and an internal rate of return (IRR) of 243%. The fluidized bioreactor process has a net present value of about \$17 billion, an ROI of about 229% and IRR of 226%. The IRR and ROI values for biotechnology projects are incredibly high due to the high market price of the product. Furthermore, drug companies rarely see a drug as successful as Rituxan®. The success of one drug helps pay for the failures of 10,000 others. A high ROI and IRR are to be expected in processes such as these due to the relatively low investment compared to the generated earnings. Furthermore, the cost of development is out-of-scope of this project and therefore not included in the economic analysis. However, each successful drug costs a company several millions of dollars and therefore the realized ROI or IRR of a biopharmaceutical process is much less than 500-600%.

13.2.1. Stirred Tank Process

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Profitability Measures

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The Internal Rate of Return (IRR) for this project is 243.03%

The Net Present Value (NPV) of this project in 2015 is \$ 3,807,362,600

ROI Analysis (Third Production Year)

Annual Sales	1,611,764,957
Annual Costs	(253,790,021)
Depreciation	(1,734,736)
Income Tax	(302,441,565)
Net Earnings	1,053,798,635
Total Capital Investment	423,677,306
ROI	248.73%

13.2.2. Fluidized Bioreactor Process

Profitability Measures

The Internal Rate of Return (IRR) for this project is	224.53%
The Net Present Value (NPV) of this project in 2015 is	\$16,625,953,100

ROI Analysis (Third Production Year)

Annual Sales	7,104,371,792
Annual Costs	(1,155,142,234)
Depreciation	(20,597,570)
Income Tax	(1,304,299,037)
Net Earnings	4,624,332,951
Total Capital Investment	2,020,253,396
ROI	228.90%

13.2.3. Price of Generic Rituximab

Currently, pricing information for Rituxan<sup>®</sup> is available for the United States market alone. Each unit of Rituxan<sup>®</sup> (assuming a unit refers to 100mg of product) is sold for about \$1450 per 100mg. The generic version can be marketed for 30% lower prices than the brand-name drug. Normally, most generic small molecule drugs are marketed at 70% lower prices. However, due to the high cost of production and the high market price of therapeutic proteins, even a 30% decrease offers a great discount to consumers. Therefore, generic rituximab will be marketed at about \$10.3 million per kg.

13.2.4. Plant Life

The new facility is expected to have a life of about 10 years. Most of the capital investment (main production units and machinery) normally has a longer lifespan, but the marketing life of the drug can be assumed to be about 10 years for a preliminary analysis. In the unfortunate case that the plant has to be shut down in as little as five years due to inability to sell generic rituximab or due to other innovations in the field, the plant will have some finite salvage value. However, for the purposes of the economic calculations, it is assumed the plant will operate for 10 years and allow two additional years for design and construction.

13.2.5. Input and Cost Summaries

13.2.5.1. Stirred Tank Process

General Information			
Process Title: <b>Monoclonal Antibody Production via Fluidized Bed Bioreactor</b>			
Product: <b>Rituximab</b>			
Plant Site Location: <b>Philadelphia, PA</b>			
Site Factor: <b>1.00</b>			
Operating Hours per Year: <b>7920</b>			
Operating Days Per Year: <b>330</b>			
Operating Factor: <b>0.9041</b>			
Product Information			
This Process will Yield			
		0	kg of Rituximab per hour
		0	kg of Rituximab per day
		152	kg of Rituximab per year
Price	\$10,262,244.00	/kg	
Chronology			

<b>Variable Cost Summary</b>		
<b><u>Variable Costs at 100% Capacity:</u></b>		
<b><u>General Expenses</u></b>		
Selling / Transfer Expenses:		\$ 15,614,004
Direct Research:		\$ 156,140,042
Allocated Research:		\$ 7,807,002
Administrative Expense:		\$ 31,228,008
Management Incentive Compensation:		\$ 19,517,505
<b>Total General Expenses</b>		<b>\$ 230,306,563</b>
<b><u>Raw Materials</u></b>	\$21,364.976000 per kg of Rituximab	<b>\$3,250,681</b>
<b><u>Byproducts</u></b>	\$0.000000 per kg of Rituximab	<b>\$0</b>
<b><u>Utilities</u></b>	\$7,533.844106 per kg of Rituximab	<b>\$1,146,274</b>
<b><u>Total Variable Costs</u></b>		<b><u>\$ 234,703,518</u></b>
<b>Fixed Cost Summary</b>		
<b><u>Operations</u></b>		
Direct Wages and Benefits		\$ 5,241,600
Direct Salaries and Benefits		\$ 786,240
Operating Supplies and Services		\$ 314,496
Technical Assistance to Manufacturing		\$ -
Control Laboratory		\$ -
<b>Total Operations</b>		<b>\$ 6,342,336</b>
<b><u>Maintenance</u></b>		
Wages and Benefits		\$ 871,240
Salaries and Benefits		\$ 217,810
Materials and Services		\$ 871,240
Maintenance Overhead		\$ 43,562
<b>Total Maintenance</b>		<b>\$ 2,003,852</b>
<b><u>Operating Overhead</u></b>		
General Plant Overhead:		\$ 505,299
Mechanical Department Services:		\$ 170,805
Employee Relations Department:		\$ 419,897
Business Services:		\$ 526,650
<b>Total Operating Overhead</b>		<b>\$ 1,622,651</b>
<b><u>Property Taxes and Insurance</u></b>		
Property Taxes and Insurance:		\$ 387,218
<b><u>Other Annual Expenses</u></b>		
Rental Fees (Office and Laboratory Space):		\$ -
Licensing Fees:		\$ -
Miscellaneous:		\$ 800,000
<b>Total Other Annual Expenses</b>		<b>\$ 800,000</b>
<b><u>Total Fixed Costs</u></b>		<b><u>\$ 11,156,057</u></b>

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**Investment Summary**


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**Bare Module Costs**

Fabricated Equipment	\$ -
Process Machinery	\$ 14,915,939
Spares	\$ -
Storage	\$ -
Other Equipment	\$ -
Catalysts	\$ -
Computers, Software, Etc.	\$ -
<b><u>Total Bare Module Costs:</u></b>	<b><u>\$ 14,915,939</u></b>

**Direct Permanent Investment**

Cost of Site Preparations:	\$ 745,797
Cost of Service Facilities:	\$ 745,797
Allocated Costs for utility plants and related facilities:	\$ -
<b><u>Direct Permanent Investment</u></b>	<b><u>\$ 16,407,533</u></b>

**Total Depreciable Capital**

Cost of Contingencies & Contractor Fees	\$ 2,953,356
<b><u>Total Depreciable Capital</u></b>	<b><u>\$ 19,360,889</u></b>

**Total Permanent Investment**

Cost of Land:	\$ 387,218
Cost of Royalties:	\$ -
Cost of Plant Start-Up:	\$ 1,936,089
<b>Total Permanent Investment - Unadjusted</b>	<b>\$ 21,684,196</b>
Site Factor	1.00
<b><u>Total Permanent Investment</u></b>	<b><u>\$ 21,684,196</u></b>

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**Working Capital**


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	<b><u>2016</u></b>
Accounts Receivable	\$ 385,002,844
Cash Reserves	\$ 945,397
Accounts Payable	\$ (1,084,181)
Rituximab Inventory	\$ 17,111,238
Raw Materials	\$ 17,812
<b>Total</b>	<b><u>\$ 401,993,110</u></b>
 <i>Present Value at 18%</i>	 \$ 340,672,127

<b><u>Total Capital Investment</u></b>	<b><u>\$ 362,356,323</u></b>
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13.2.5.2. Fluidized Bioreactor Process

General Information					
Process Title:	Monoclonal Antibody Production via Fluidized Bed Bioreactor				
Product:	Rituximab				
Plant Site Location:	Philadelphia, PA				
Site Factor:	1.00				
Operating Hours per Year:	7920				
Operating Days Per Year:	330				
Operating Factor:	0.9041				
Product Information					
This Process will Yield					
	0 kg of Rituximab per hour				
	2 kg of Rituximab per day				
	671 kg of Rituximab per year				
Price	\$10,262,244.00 /kg				
Chronology					
Year	Action	Distribution of Permanent Investment	Production Capacity	Depreciation 7 year MACRS	Product Price
2015	Design		0.0%		
2016	Construction	100%	0.0%		
2017	Production	0%	100.0%	14.29%	\$10,262,244.00
2018	Production	0%	100.0%	24.49%	\$10,426,439.90
2019	Production	0%	100.0%	17.49%	\$10,593,262.94
2020	Production		100.0%	12.49%	\$10,762,755.15
2021	Production		100.0%	8.93%	\$10,934,959.23
2022	Production		100.0%	8.92%	\$11,109,918.58
2023	Production		100.0%	8.93%	\$11,287,677.28
2024	Production		100.0%	4.46%	\$11,468,280.11
2025	Production		100.0%		\$11,651,772.60
2026	Production		100.0%		\$11,838,200.96

<b>Variable Cost Summary</b>			
<b><u>Variable Costs at 100% Capacity:</u></b>			
<b><u>General Expenses</u></b>			
	Selling / Transfer Expenses:	\$	68,823,739
	Direct Research:	\$	688,237,394
	Allocated Research:	\$	34,411,870
	Administrative Expense:	\$	137,647,479
	Management Incentive Compensation:	\$	86,029,674
	<b>Total General Expenses</b>		<b>\$1,015,150,156</b>
<b><u>Raw Materials</u></b>	\$81,786.793414 per kg of Rituximab		<b>\$54,850,313</b>
<b><u>Byproducts</u></b>	\$0.000000 per kg of Rituximab		<b>\$0</b>
<b><u>Utilities</u></b>	\$2,569.985019 per kg of Rituximab		<b>\$1,723,560</b>
<b><u>Total Variable Costs</u></b>			<b><u>\$1,071,724,029</u></b>
<b>Fixed Cost Summary</b>			
<b><u>Operations</u></b>			
	Direct Wages and Benefits	\$	10,483,200
	Direct Salaries and Benefits	\$	1,572,480
	Operating Supplies and Services	\$	628,992
	Technical Assistance to Manufacturing	\$	-
	Control Laboratory	\$	-
	<b>Total Operations</b>	<b>\$</b>	<b>12,684,672</b>
<b><u>Maintenance</u></b>			
	Wages and Benefits	\$	10,344,762
	Salaries and Benefits	\$	2,586,190
	Materials and Services	\$	10,344,762
	Maintenance Overhead	\$	517,238
	<b>Total Maintenance</b>	<b>\$</b>	<b>23,792,952</b>
<b><u>Operating Overhead</u></b>			
	General Plant Overhead:	\$	1,774,051
	Mechanical Department Services:	\$	599,679
	Employee Relations Department:	\$	1,474,211
	Business Services:	\$	1,849,011
	<b>Total Operating Overhead</b>	<b>\$</b>	<b>5,696,952</b>
<b><u>Property Taxes and Insurance</u></b>			
	Property Taxes and Insurance:	\$	4,597,672
<b><u>Other Annual Expenses</u></b>			
	Rental Fees (Office and Laboratory Space):	\$	-
	Licensing Fees:	\$	-
	Miscellaneous:	\$	550,000
	<b>Total Other Annual Expenses</b>	<b>\$</b>	<b>550,000</b>
<b><u>Total Fixed Costs</u></b>		<b>\$</b>	<b><u>47,322,248</u></b>

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**Investment Summary**


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**Bare Module Costs**

Fabricated Equipment	\$	-
Process Machinery	\$	177,106,004
Spares	\$	-
Storage	\$	-
Other Equipment	\$	-
Catalysts	\$	-
Computers, Software, Etc.	\$	-
<b><u>Total Bare Module Costs:</u></b>	<b>\$</b>	<b><u>177,106,004</u></b>

**Direct Permanent Investment**

Cost of Site Preparations:	\$	8,855,300
Cost of Service Facilities:	\$	8,855,300
Allocated Costs for utility plants and related facilities:	\$	-
<b><u>Direct Permanent Investment</u></b>	<b>\$</b>	<b><u>194,816,605</u></b>

**Total Depreciable Capital**

Cost of Contingencies & Contractor Fees	\$	35,066,989
<b><u>Total Depreciable Capital</u></b>	<b>\$</b>	<b><u>229,883,593</u></b>

**Total Permanent Investment**

Cost of Land:	\$	4,597,672
Cost of Royalties:	\$	-
Cost of Plant Start-Up:	\$	22,988,359
<b>Total Permanent Investment - Unadjusted</b>	<b>\$</b>	<b>257,469,625</b>
Site Factor		1.00
<b><u>Total Permanent Investment</u></b>	<b>\$</b>	<b><u>257,469,625</u></b>

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**Working Capital**


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	<b><u>2016</u></b>
Accounts Receivable	\$ 1,697,023,711
Cash Reserves	\$ 3,985,957
Accounts Payable	\$ (13,949,722)
Rituximab Inventory	\$ 75,423,276
Raw Materials	\$ 300,550
<b>Total</b>	<b><u>\$ 1,762,783,771</u></b>
<i>Present Value at 18%</i>	<i>\$ 1,493,884,552</i>
<b><u>Total Capital Investment</u></b>	<b><u>\$ 1,751,354,176</u></b>

### **13.2.6. Fixed Costs**

For both designs, the plant will run for 24 hours and require 3 total shifts. Each shift for the stirred tank process will have 20 operators and each shift for the fluidized bed reactor will have 40 operators. Because this is a brownfield site, annual expenses were estimated to include the cost of using the site's existing equipment such as quality control labs, waste treatment systems, and WFI stills. The operators will have direct wages and benefits totaling \$42.00/hr. Engineers and scientists will be placed throughout the facility for the production of monoclonal antibody. They will work with the seed train, production, and purification process and will operate all the machinery. There will also be people working in the culture labs and managing the material, for example measuring media, buffer, etc. For the fluidized bed process, many more workers are needed per shift in order to manage all of the media and buffer prep solutions. There needs to be maintenance and utility personnel, IT and auto control personnel, and people to manage quality control, CIP & SIP procedures, and waste management. Again, additional workers are needed to manage the fluidized bed facility because it is larger in surface area due to the increased number of storage tanks and pumps. For the facilities, regulatory personnel are also needed. Lastly, management positions will be needed to manage the engineering, financial, and human resources related issues.

13.2.6.1. *Stirred Tank Process*

<b>Fixed Costs</b>		
<u>Operations</u>		
Operators per Shift:	20	(assuming 3 shifts)
Direct Wages and Benefits:	\$42	/operator hour
Direct Salaries and Benefits:	15%	of Direct Wages and Benefits
Operating Supplies and Services:	6%	of Direct Wages and Benefits
Technical Assistance to Manufacturing:	\$0.00	per year, for each Operator per Shift
Control Laboratory:	\$0.00	per year, for each Operator per Shift
<u>Maintenance</u>		
Wages and Benefits:	4.50%	of Total Depreciable Capital
Salaries and Benefits:	25.00%	of Maintenance Wages and Benefits
Materials and Services:	100.00%	of Maintenance Wages and Benefits
Maintenance Overhead:	5.00%	of Maintenance Wages and Benefits
<u>Operating Overhead</u>		
General Plant Overhead:	7.10%	of Maintenance and Operations Wages and Benefits
Mechanical Department Services:	2.40%	of Maintenance and Operations Wages and Benefits
Employee Relations Department	5.90%	of Maintenance and Operations Wages and Benefits
Business Services	7.40%	of Maintenance and Operations Wages and Benefits
<u>Property Taxes and Insurance</u>		
Property Taxes and Insurance:	2.00%	of Total Depreciable Capital
<u>Straight Line Depreciation</u>		
Direct Plant:	8.00%	of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities
Allocated Plant:	6.00%	of 1.18 times the Allocated Costs for Utility Plants and Related Facilities
<u>Other Annual Expenses</u>		
Rental Fees (Office and Laboratory Space):	\$0	
Licensing Fees:	\$0	
Miscellaneous:	\$800,000	
<u>Depletion Allowance</u>		
Annual Depletion Allowance:	\$0	

**13.2.6.2. Fluidized Bioreactor Process**

<b>Fixed Costs</b>		
<b><u>Operations</u></b>		
Operators per Shift:	40	(assuming 3 shifts)
Direct Wages and Benefits:	\$42	/operator hour
Direct Salaries and Benefits:	15%	of Direct Wages and Benefits
Operating Supplies and Services:	6%	of Direct Wages and Benefits
Technical Assistance to Manufacturing:	\$0.00	per year, for each Operator per Shift
Control Laboratory:	\$0.00	per year, for each Operator per Shift
<b><u>Maintenance</u></b>		
Wages and Benefits:	4.50%	of Total Depreciable Capital
Salaries and Benefits:	25.00%	of Maintenance Wages and Benefits
Materials and Services:	100.00%	of Maintenance Wages and Benefits
Maintenance Overhead:	5.00%	of Maintenance Wages and Benefits
<b><u>Operating Overhead</u></b>		
General Plant Overhead:	7.10%	of Maintenance and Operations Wages and Benefits
Mechanical Department Services:	2.40%	of Maintenance and Operations Wages and Benefits
Employee Relations Department	5.90%	of Maintenance and Operations Wages and Benefits
Business Services	7.40%	of Maintenance and Operations Wages and Benefits
<b><u>Property Taxes and Insurance</u></b>		
Property Taxes and Insurance:	2.00%	of Total Depreciable Capital
<b><u>Straight Line Depreciation</u></b>		
Direct Plant:	8.00%	of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities
Allocated Plant:	6.00%	of 1.18 times the Allocated Costs for Utility Plants and Related Facilities
<b><u>Other Annual Expenses</u></b>		
Rental Fees (Office and Laboratory Space):	\$0	
Licensing Fees:	\$0	
Miscellaneous:	\$550,000	
<b><u>Depletion Allowance</u></b>		
Annual Depletion Allowance:	\$0	

**13.2.7. Raw Materials and Equipment Costs**

Equipment costs were combined for similar units. The bare module cost was assumed to be 3.21 for all equipment.

**Bare Module Factor Calculator:**

Cost of Installation Materials:	<b>71%</b>	of Equipment Purchase Cost
Cost of Installation Labor:	<b>54%</b>	of Equipment Purchase Cost
Cost for Freight, Insurances, and Taxes:	<b>9%</b>	of Equipment Purchase Cost
Cost of Construction Overhead:	<b>57%</b>	of Equipment Purchase Cost
Cost of Contractor Engineering Expenses:	<b>30%</b>	of Equipment Purchase Cost
<hr/>		
Total Derived Bare Module Factor:	<b><u>3.21</u></b>	of Equipment Purchase Cost

13.2.7.1. *Stirred Tank Process*

Raw Materials				
Raw Material:	Unit:	Required Ratio:		Cost of Raw Material:
1 Resin	L	3.947	L per kg of Rituximab	\$4000.000 per L
2 Media	kg	52.755	kg per kg of Rituximab	\$35.200 per kg
3 Rituximab	kg	1	kg per kg of Rituximab	\$0.000E+00 per kg
4 Buffer	kg	3000	kg per kg of Rituximab	\$1.240 per kg
5 Microcarriers	kg	0	kg per kg of Rituximab	\$4488.000 per kg
6				
7				
8				
9				
10				
Total Weighted Average:				\$21364.976 per kg of Rituximab

Equipment Costs

Equipment Description	Type	Purchase Cost	Bare Module Cost
Name			
PID Controls	Process Machinery	\$162,500	\$521,625
Bioreactor Rocker	Process Machinery	\$1,020,980	\$3,277,346
Pump	Process Machinery	\$299,000	\$959,790
Mixing Tank	Process Machinery	\$260,230	\$835,338
Sterile Filter	Process Machinery	\$10,000	\$32,100
Ultrafiltration Unit	Process Machinery	\$120,000	\$385,200
Production Bioreactor	Process Machinery	\$900,000	\$2,889,000
Centrifuge	Process Machinery	\$480,000	\$1,540,800
Storage Tank	Process Machinery	\$679,000	\$2,179,590
Protein A Column	Process Machinery	\$200,000	\$642,000
Cation Ex Column	Process Machinery	\$250,000	\$802,500
Anion Ex Column	Process Machinery	\$250,000	\$802,500
Nanofiltration Unit	Process Machinery	\$15,000	\$48,150

13.2.7.2. *Fluidized Bioreactor Process*

Raw Materials				
Raw Material:	Unit:	Required Ratio:		Cost of Raw Material:
1 Resin	L	0.895	L per kg of Rituximab	\$4000.000 per L
2 Media	kg	3080.597	kg per kg of Rituximab	\$0.035 per kg
3 Rituximab	kg	1	kg per kg of Rituximab	\$0.000E+00 per kg
4 Buffer	kg	517.91	kg per kg of Rituximab	\$1.240 per kg
5 Microcarriers	kg	17.2585	kg per kg of Rituximab	\$4488.000 per kg
6				
7				
8				
9				
10				
Total Weighted Average:				\$81786.793 per kg of Rituximab

### Equipment Costs

<u>Equipment Description</u> Name	<u>Type</u>	<u>Purchase Cost</u>	<u>Bare Module Cost</u>
PID Controls	Process Machinery	\$1,620,000	\$5,200,200
Bioreactor Rocker	Process Machinery	\$3,329,980	\$10,689,236
Pump	Process Machinery	\$2,444,000	\$7,845,240
Mixing Tank	Process Machinery	\$405,230	\$1,300,788
Sterile Filter	Process Machinery	\$10,000	\$32,100
Ultrafiltration Unit	Process Machinery	\$72,000	\$385,200
Fluidized Bioreactor	Process Machinery	\$1,500,000	\$4,815,000
Tangential Flow Filtration	Process Machinery	\$25,000,000	\$80,250,000
Storage Tanks	Process Machinery	\$19,529,000	\$62,688,090
Protein A Column	Process Machinery	\$200,000	\$642,000
Cation Ex Column	Process Machinery	\$250,000	\$802,500
Anion Ex Column	Process Machinery	\$250,000	\$802,500
Nanofiltration Unit	Process Machinery	\$15,000	\$48,150
Autoclave Sterilizer	Process Machinery	\$500,000	\$1,605,000

### 13.2.8. Other Variable Costs and Working Capital

For the purposes of working capital, it was assumed that about one percent of sales would be spent on selling/transfer costs paid to a sister facility. Ten percent of sales would be recycled back into research and development. Furthermore, the accounts payable and receivable periods were extended to 90 days.

#### 13.2.8.1. Stirred Tank Process

#### Other Variable Costs

##### General Expenses

Selling / Transfer Expenses:	1.00% of Sales
Direct Research:	10.00% of Sales
Allocated Research:	0.50% of Sales
Administrative Expense:	2.00% of Sales
Management Incentive Compensation:	1.25% of Sales

#### Working Capital

Accounts Receivable	⇒	90 Days
Cash Reserves (excluding Raw Materials)	⇒	30 Days
Accounts Payable	⇒	90 Days
Rituximab Inventory	⇒	4 Days
Raw Materials	⇒	2 Days



**13.2.8.2. Fluidized Bioreactor Process****Other Variable Costs****General Expenses**

Selling / Transfer Expenses:	1.00%	of Sales
Direct Research:	10.00%	of Sales
Allocated Research:	0.50%	of Sales
Administrative Expense:	2.00%	of Sales
Management Incentive Compensation:	1.25%	of Sales

**Working Capital**

Accounts Receivable	⇒	90 Days
Cash Reserves (excluding Raw Materials)	⇒	30 Days
Accounts Payable	⇒	90 Days
Rituximab Inventory	⇒	4 Days
Raw Materials	⇒	2 Days

**13.2.9. Utilities**

As described in the previous section 10, large amounts of process water and electricity will be required for both processes.

**13.2.9.1. Stirred Tank Process****Utilities**

Utility:	Unit:	Required Ratio	Utility Cost
1 High Pressure Steam	lb	0 lb per kg of Rituximab	per lb
2 Low Pressure Steam	lb	0 lb per kg of Rituximab	\$0.000E+00 per lb
3 Process Water	kg	20368.73275 kg per kg of Rituximab	\$4.730E-03 per kg
4 Cooling Water	lb	0 lb per kg of Rituximab	per lb
5 Electricity	kWh	8.75E+04 kWh per kg of Rituximab	\$0.085 per kWh
6			
7			
8			
9			
10			
Total Weighted Average:			\$7533.844 per kg of Rituximab

**13.2.9.2. Fluidized Bioreactor Process****Utilities**

Utility:	Unit:	Required Ratio	Utility Cost
1 High Pressure Steam	lb	0 lb per kg of Rituximab	per lb
2 Low Pressure Steam	lb	0 lb per kg of Rituximab	\$0.000E+00 per lb
3 Process Water	kg	157107.69 kg per kg of Rituximab	\$4.730E-03 per kg
4 Cooling Water	lb	0 lb per kg of Rituximab	per lb
5 Electricity	kWh	2.15E+04 kWh per kg of Rituximab	\$0.085 per kWh
6			
7			
8			
9			
10			
Total Weighted Average:			\$2569.985 per kg of Rituximab

### 13.3. Sensitivity Analysis

A sensitivity analysis must be conducted to determine the effect of key variables on the profitability of a plant. Furthermore, a sensitivity analysis will help determine which process design is superior.

#### 13.3.1. Variable Cost

In the base cases for both designs, the variable costs for the stirred tank process are about \$235 million and about \$1 billion for the fluidized bioreactor. There is a significant difference between the two cases because variable costs were calculated as a percentage of products produced. In terms of variable costs per kg, the stirred tank costs \$1.5 million per kg while the fluidized bioreactor costs \$1.6 million per kg. The fluidized bioreactor process does have a higher variable cost to produce the same amount of product. However, other factors such as total permanent investment, product price, and ultimately the cash flow from revenue can affect the profitability of a project. Furthermore, changes in product price and variable costs, which may positively or negatively impact revenue, can drastically change the internal rate of return for the processes. For both processes, the internal rates of return are extremely high, but can yield negative IRR values for low variable costs and low product prices.

**Table 13.2:** The effect of variable costs and product prices on the internal rate of return of the stirred tank process.

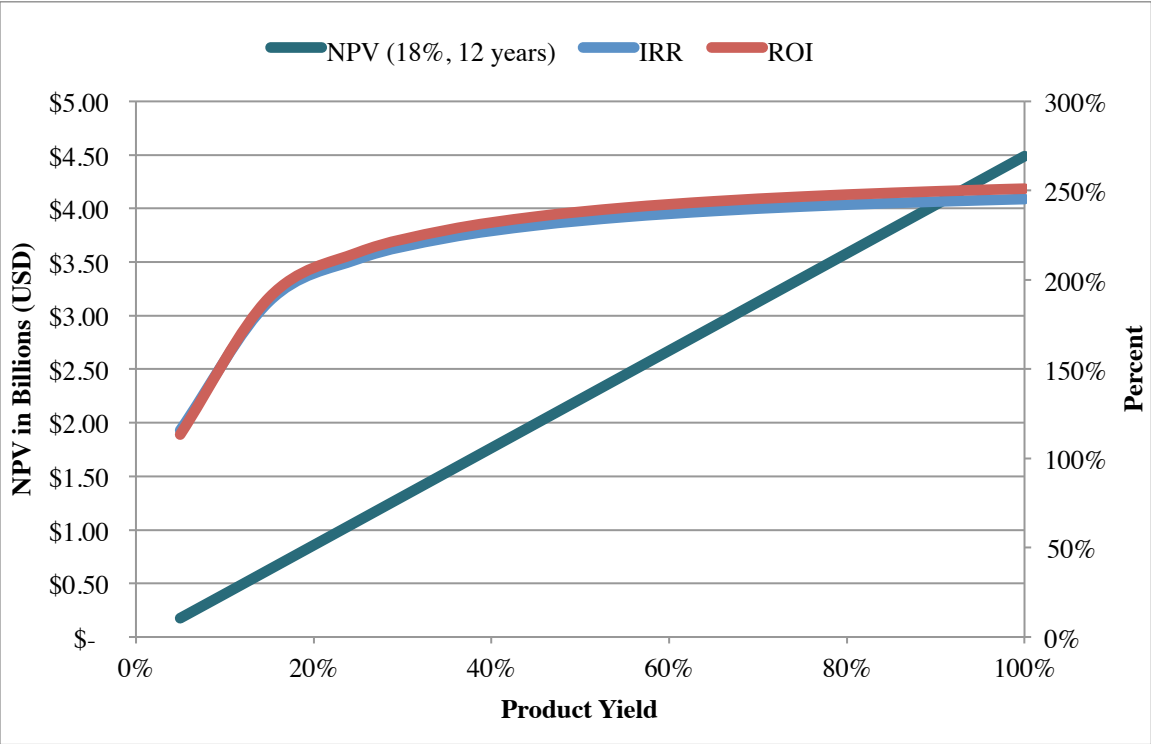
Sensitivity Analyses											
Product Price	Variable Costs										
	\$2,347,035	\$48,818,332	\$95,289,628	\$141,760,925	\$188,232,222	<b>\$234,703,518</b>	\$281,174,815	\$327,646,111	\$374,117,408	\$420,588,704	\$467,060,001
	\$102,622	0.08%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	\$2,134,547	205.86%	175.45%	145.05%	114.63%	84.16%	53.53%	22.12%	-12.92%	Negative IRR	Negative IRR
	\$4,166,471	249.20%	230.68%	212.16%	193.64%	175.13%	156.61%	138.09%	119.56%	101.03%	82.47%
	\$6,198,395	268.17%	254.86%	241.55%	228.23%	214.92%	201.61%	188.30%	174.98%	161.67%	148.35%
	\$8,230,320	278.82%	268.43%	258.04%	247.65%	237.26%	226.86%	216.47%	206.08%	195.69%	185.29%
	<b>\$10,262,244</b>	285.64%	277.12%	268.60%	260.07%	251.55%	243.03%	234.51%	225.98%	217.46%	208.94%
	\$12,294,168	290.38%	283.16%	275.93%	268.71%	261.49%	254.27%	247.04%	239.82%	232.60%	225.37%
	\$14,326,093	293.86%	287.60%	281.33%	275.06%	268.80%	262.53%	256.26%	249.99%	243.73%	237.46%
	\$16,358,017	296.53%	291.00%	285.47%	279.93%	274.40%	268.86%	263.33%	257.79%	252.26%	246.72%
	\$18,389,941	298.65%	293.69%	288.73%	283.78%	278.82%	273.87%	268.91%	263.96%	259.00%	254.04%
	\$20,421,866	300.36%	295.87%	291.38%	286.90%	282.41%	277.93%	273.44%	268.95%	264.47%	259.98%

**Table 13.3:** The effect of variable costs and product prices on the internal rate of return of the fluidized bioreactor process.

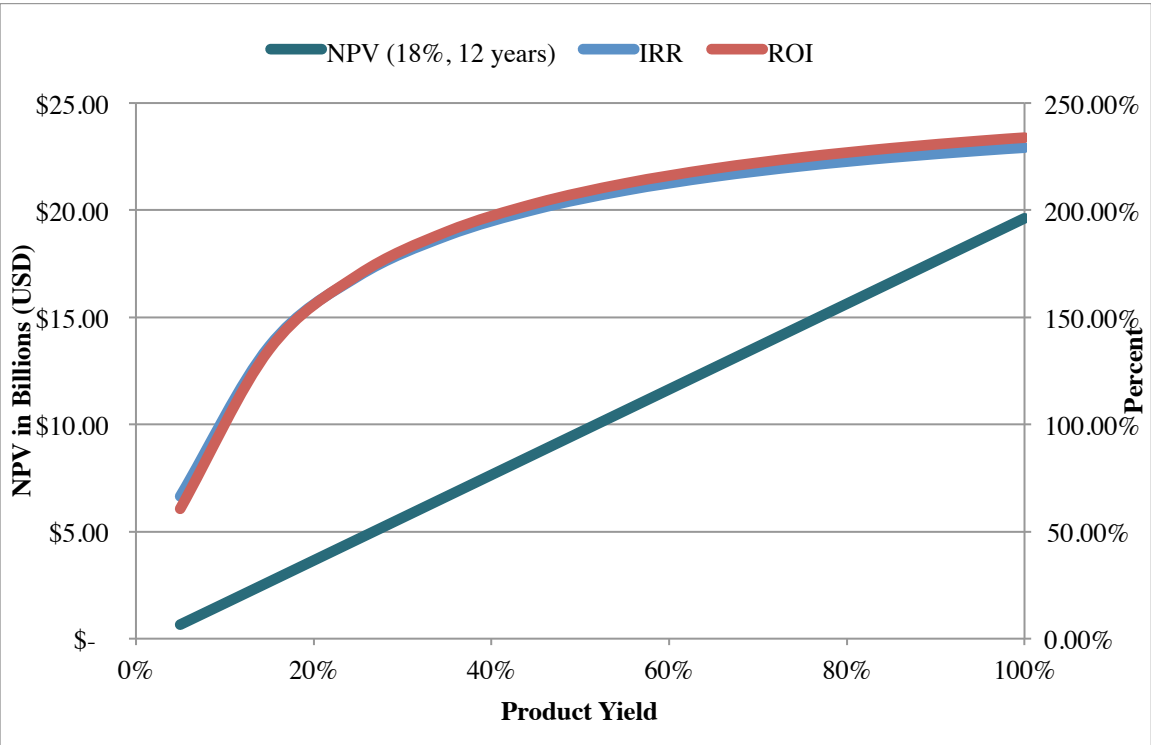
Sensitivity Analyses											
Product Price	Variable Costs										
	\$10,717,240	\$222,918,598	\$435,119,956	\$647,321,314	\$859,522,672	\$1,071,724,029	\$1,283,925,387	\$1,496,126,745	\$1,708,328,103	\$1,920,529,461	\$2,132,730,819
	\$102,622	-5.73%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
	\$2,134,547	161.27%	136.79%	112.28%	87.73%	63.04%	37.87%	10.87%	Negative IRR	Negative IRR	Negative IRR
	\$4,166,471	213.27%	196.92%	180.57%	164.22%	147.86%	131.51%	115.14%	98.76%	82.36%	65.90%
	\$6,198,395	239.35%	227.08%	214.80%	202.53%	190.25%	177.97%	165.70%	153.42%	141.14%	128.86%
	\$8,230,320	255.03%	245.20%	235.37%	225.55%	215.72%	205.90%	196.07%	186.24%	176.41%	166.59%
	<b>\$10,262,244</b>	265.49%	257.30%	249.11%	240.91%	232.72%	224.53%	216.34%	208.14%	199.95%	191.76%
	\$12,294,168	272.97%	265.94%	258.92%	251.90%	244.87%	237.85%	230.82%	223.80%	216.77%	209.75%
	\$14,326,093	278.58%	272.43%	266.28%	260.14%	253.99%	247.84%	241.69%	235.55%	229.40%	223.25%
Product Price	\$16,358,017	282.95%	277.48%	272.01%	266.55%	261.08%	255.62%	250.15%	244.69%	239.22%	233.76%
	\$18,389,941	286.44%	281.52%	276.60%	271.68%	266.76%	261.84%	256.92%	252.00%	247.08%	242.16%
	\$20,421,866	289.30%	284.83%	280.35%	275.88%	271.41%	266.94%	262.46%	257.99%	253.52%	249.04%

### 13.3.2. Overall Product Yield

In the base case, it is assumed that 85% of the product will be recovered after downstream purification. However, technical errors or errors in process design may often lower the yield of a process. Assuming only the yield changes but all other costs remain constant, product yield can dramatically change the profitability of a project. For the stirred tank process, in the lower limit of only 5% yield, the NPV is about \$175 million, the IRR and ROI are about 115%. In the upper limit of complete product recovery, the ROI and IRR are approximately 250% and the NPV is about \$4.5 billion. On the other hand, the range of 5% to 100% product yield for the fluidized bioreactor process yields an NPV range of \$654 million to \$19.6 billion and IRR and ROI ranges of 65% to 230%. For all product yields, the fluidized bioreactor process is the fiscally superior process.



**Figure 13.1:** The impact of product yield on net present value of project, the internal rate of return, and the return on investment for the stirred tank process.

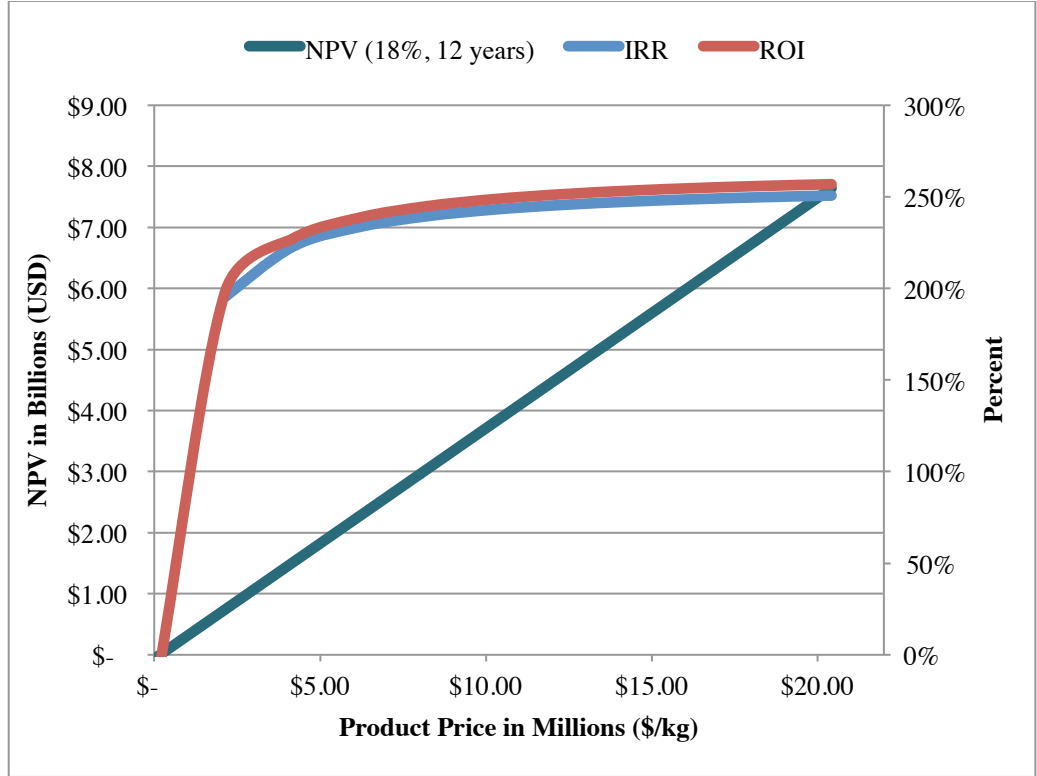


**Figure 13.2:** The impact of product yield on net present value of project, the internal rate of return, and the return on investment for the fluidized bioreactor process.

13.3.3. Product Price

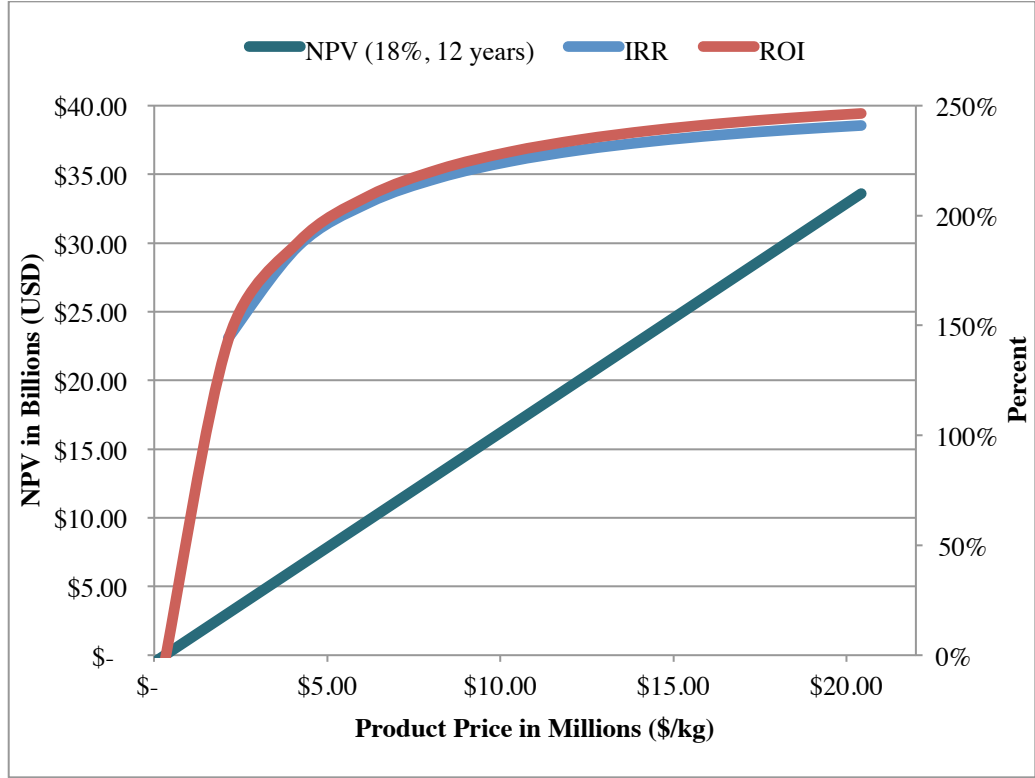
In the base case, it is assumed that the product will be sold for about \$10.3MM/kg. Due to the unpredictability of pharmaceutical markets, the product’s selling price may change due to an increase in process costs or a decrease in the demand for the drug. For the stirred tank process, given the range of product price to be \$100,000/kg to \$20.5MM/kg, the NPV ranges from -\$26MM to \$7.6 billion and the IRR and ROI values range from negative values to 255%. For the fluidized bioreactor process, the NPV ranges from -\$340MM to \$34 billion and the IRR and ROI values range from negative values to 245%. In the case that the product price is greater than \$105,000/kg, the fluidized bioreactor process is more favorable.

13.3.3.1. Stirred Tank Process



**Figure 13.3:** The impact of product price on net present value of project, the internal rate of return, and the return on investment for stirred tank process.

13.3.3.2. Fluidized Bioreactor Process



**Figure 13.4:** The impact of product price on net present value of project, the internal rate of return, and the return on investment for the fluidized bioreactor process.

13.3.4. Plant Life

In the base case, it is assumed the plant has an operational life of 10 years. For both processes, if as the life span increases, the NPV also increases but then levels out to an approximately constant value. Regardless of plant life, the fluidized bioreactor process has a significantly larger NPV.

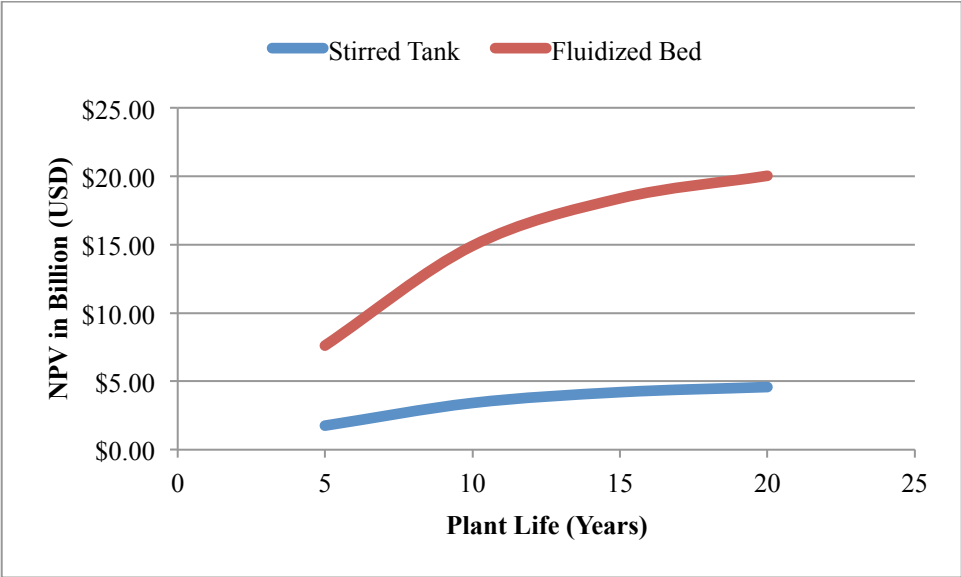


Figure 13.5: The impact of plant life on the net present value of the project for both processes.

13.3.5. Cost of Capital

The cost of capital determines the riskiness of a project and gives a minimum value of return required for a project to be acceptable. The cost of capital of the project is assumed to be 18% for the base case. However, as the cost of capital increases, the NPV of the project will decrease. Regardless of the cost of capital, the fluidized bioreactor consistently has a significantly higher NPV of the project.

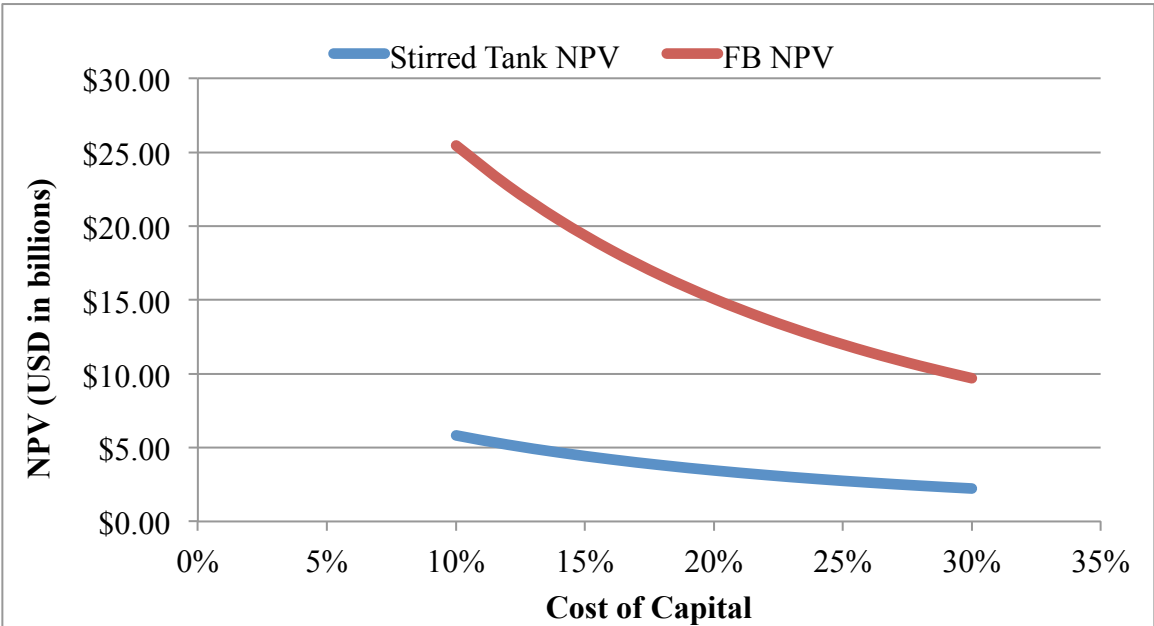


Figure 13.6: The impact of cost of capital on the net present value of the project for both processes.

### 13.3.6. Differential Present Worth Analysis

To ultimately determine which process is preferable the after-tax cash flows have to be analyzed for both projects. The after-tax cash flow includes capital costs, working capital, variable and fixed costs, depreciation, and taxes.

**Table 13.4:** The after-tax cash flow for the stirred tank process.

Year	Sales	Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Taxible Income	Taxes	Cash Flow
2015	-	-	-	-	-	-	-	-	-
2016	-	(21,684,196)	(401,993,100)	-	-	-	-	-	(423,677,306)
2017	1,561,400,400	-	-	(234,703,518)	(11,156,057)	(2,766,700)	1,312,774,200	(292,748,642)	1,022,792,208
2018	1,586,382,800	-	-	(238,458,774)	(11,334,554)	(4,741,500)	1,331,848,000	(297,002,109)	1,039,587,395
2019	1,611,765,000	-	-	(242,274,115)	(11,515,906)	(3,386,200)	1,354,588,700	(302,073,284)	1,055,901,652
2020	1,637,553,200	-	-	(246,150,501)	(11,700,161)	(2,418,200)	1,377,284,400	(307,134,412)	1,072,568,122
2021	1,663,754,000	-	-	(250,088,909)	(11,887,364)	(1,728,900)	1,400,048,800	(312,210,893)	1,089,566,882
2022	1,690,374,100	-	-	(254,090,331)	(12,077,561)	(1,727,000)	1,422,479,200	(317,212,868)	1,106,993,351
2023	1,717,420,100	-	-	(258,155,776)	(12,270,802)	(1,728,900)	1,445,264,600	(322,294,004)	1,124,699,515
2024	1,744,898,800	-	-	(262,286,269)	(12,467,135)	(863,500)	1,469,281,900	(327,649,868)	1,142,495,547
2025	1,772,817,200	-	-	(266,482,849)	(12,666,609)	-	1,493,667,700	(333,087,906)	1,160,579,835
2026	1,801,182,300	-	401,993,100	(270,746,575)	(12,869,275)	-	1,517,566,400	(338,417,313)	1,581,142,223

**Table 13.5:** The after-tax cash flow for the stirred tank process.

Year	Sales	Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Taxible Income	Taxes	Cash Flow
2015	-	-	-	-	-	-	-	-	-
2016	-	(257,469,625)	(1,762,783,800)	-	-	-	-	-	(2,020,253,396)
2017	6,882,373,900	-	-	(1,071,724,029)	(47,322,248)	(32,850,400)	5,730,477,300	(1,260,705,005)	4,502,622,656
2018	6,992,491,900	-	-	(1,088,871,614)	(48,079,404)	(56,298,500)	5,799,242,400	(1,275,833,331)	4,579,707,573
2019	7,104,371,800	-	-	(1,106,293,560)	(48,848,674)	(40,206,600)	5,909,022,900	(1,299,985,042)	4,649,244,516
2020	7,218,041,700	-	-	(1,123,994,257)	(49,630,253)	(28,712,500)	6,015,704,800	(1,323,455,050)	4,720,962,182
2021	7,333,530,400	-	-	(1,141,978,165)	(50,424,337)	(20,528,600)	6,120,599,300	(1,346,531,846)	4,794,596,061
2022	7,450,866,900	-	-	(1,160,249,815)	(51,231,127)	(20,505,600)	6,218,880,300	(1,368,153,674)	4,871,232,279
2023	7,570,080,800	-	-	(1,178,813,812)	(52,050,825)	(20,528,600)	6,318,687,500	(1,390,111,255)	4,949,104,873
2024	7,691,202,100	-	-	(1,197,674,833)	(52,883,638)	(10,252,800)	6,430,390,800	(1,414,685,971)	5,025,957,616
2025	7,814,261,300	-	-	(1,216,837,631)	(53,729,776)	-	6,543,693,900	(1,439,612,655)	5,104,081,230
2026	7,939,289,500	-	1,762,783,800	(1,236,307,033)	(54,589,452)	-	6,648,393,000	(1,462,646,457)	6,948,530,301

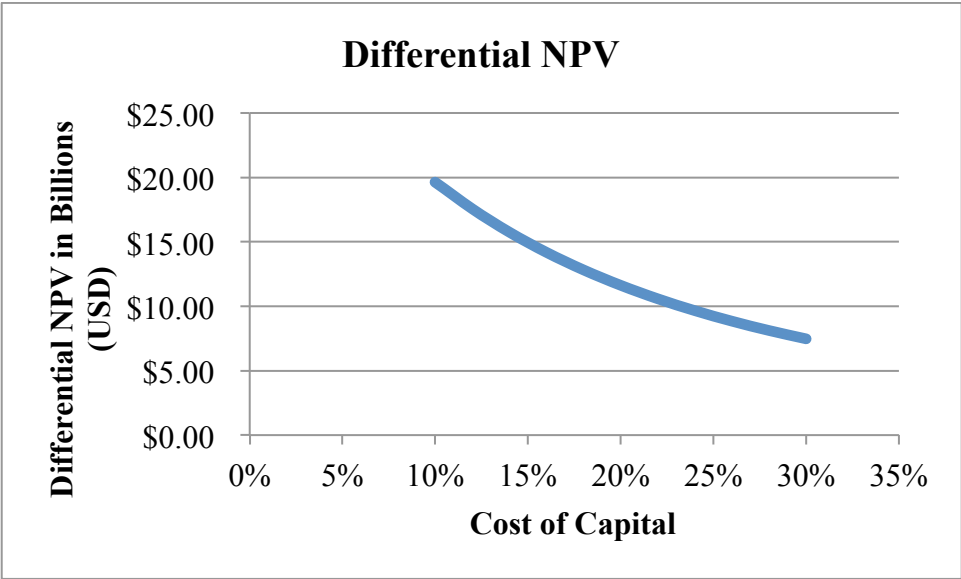
Next, the stirred tank process is chosen as the “base case” because it has the lowest initial investment. The fluidized bioreactor process is chosen as the “alternative.” For the years 2015 to 2026, the cash flow of the stirred tank process was subtracted from the cash flow for the fluidized bioreactor process.



**Table 13.6:** The after-tax cash flow for the stirred tank process.

Year	(FB-Tank) Cash Flows
2015	\$ -
2016	\$ (1,596,576,090)
2017	\$ 3,479,830,448
2018	\$ 3,540,120,179
2019	\$ 3,593,342,865
2020	\$ 3,648,394,060
2021	\$ 3,705,029,179
2022	\$ 3,764,238,928
2023	\$ 3,824,405,359
2024	\$ 3,883,462,068
2025	\$ 3,943,501,394
2026	\$ 5,367,388,078

Then, the net present value of the differential cash flow amounts is calculated to determine which process is more favorable. The NPV was calculated for costs of capital ranging from 5% to 30%. For all values, the differential NPV was extremely positive indicating the fluidized bioreactor is the more favorable process.



**Figure 13.7:** Differential net present value of the difference in cash flows between the two processes for varying costs of capital.

## **14. Additional Consideration**

Along with creating a cost-effective design to produce rituximab, FDA regulations and good manufacturing practices (GMP) must always be followed. Furthermore, plants of this caliber will have significant waste requirements and all disposal must also abide by the rules instated by the Environmental Protection Agency.

### **14.1. Environmental Concerns**

The monoclonal antibody production process creates biological and non-biological waste. First, several disposable units are used throughout the process which must be sterilized and disposed properly. Second, a variety of buffers are also used throughout downstream purification. Third, a significant amount of biowaste is generated. Any streams containing living cells and viruses are considered class II biohazards and must be treated by the biowaste inactivation package. This package will heat the waste to 80°C for one minute and inactivate the CHO cells. After inactivation, the waste is combined with non-biological waste in the waste neutralization tank (which neutralizes content pH to 7.0). This waste can then be safely disposed into the sewage system.

All disposable bags and filter membranes must be autoclaved or sterilized (if autoclaving is not possible for a certain unit) to thoroughly kill all cells and toxins. A contract waste disposal facility will be responsible for handling all landfill waste. Unfortunately, recycling services still do not exist for disposable bag bioreactors, which results in a significant carbon footprint for the plant.

### **14.2. Current Good Manufacturing Practices**

Following good manufacturing practices, as set forth by the United States Food and Drug Administration (FDA), quality materials and units must be used, a strictly regulated and well-trained staff must be on the floor at all times to monitor plant and product safety, and the plant layout must be approved. To help ensure that product from every batch is consistently safe, operators will monitor various quality checkpoints throughout the process. These checkpoints are usually placed strategically throughout the process, especially at key decision points. For example, there will be in-process quality checks after each chromatography column as well as a check at the end of the production process. Several other quality checks will also be routinely performed. A final quality check will be

performed at the end of the purification process before the drug is shipped to a contract facility for final packaging and shipping.

All quality checks involve removing small samples from transfer streams and testing the samples in a quality control lab. Every check is documented and follows a validation protocol; these documents are stored to provide reference for the manufacturer and the FDA (refer to FDA's Code of Federal Regulations Title 21, Subchapter C). Furthermore, sterility throughout the process is maintained by using standard CIP and SIP techniques (see Appendix G). As discussed before, disposable units such as the bag bioreactors help eliminate cross contamination and increase the sterility of the process.

It is also important to note that changes in facility design or innovative facility ideas such as the fluidized bioreactor design require FDA approval before operating. In general, the FDA will analyze new designs and facilities on the basis of maintain product integrity. As long as sufficient trials can prove the consistency and safety of a design and as long as the process can produce the desired drug at the mandated purity level, a new design can receive FDA approval.

### **14.3. Plant Layout**

Designing a plant is equally as important as designing the process. In a biopharmaceutical plant, all rooms must comply with stringent quality standards in order to avoid cross contamination during operation. Figure 14.1 shows a sample plant layout. The manufacturing and purification chambers are completely separated. A separate room is used for the cell bank and preparatory areas. Finally, quality control has been placed at the far side of the plant to ensure ample testing space and to decrease the any possibility of sample contamination. Similarly, the autoclave and waste area rooms are located further away from the main process area. In general, the plant will be equipped with air locks where needed, include air purification filters in each room, and include a closed layout so that each room can be completely isolated from the remainder of the plant in case of emergencies or spillage.

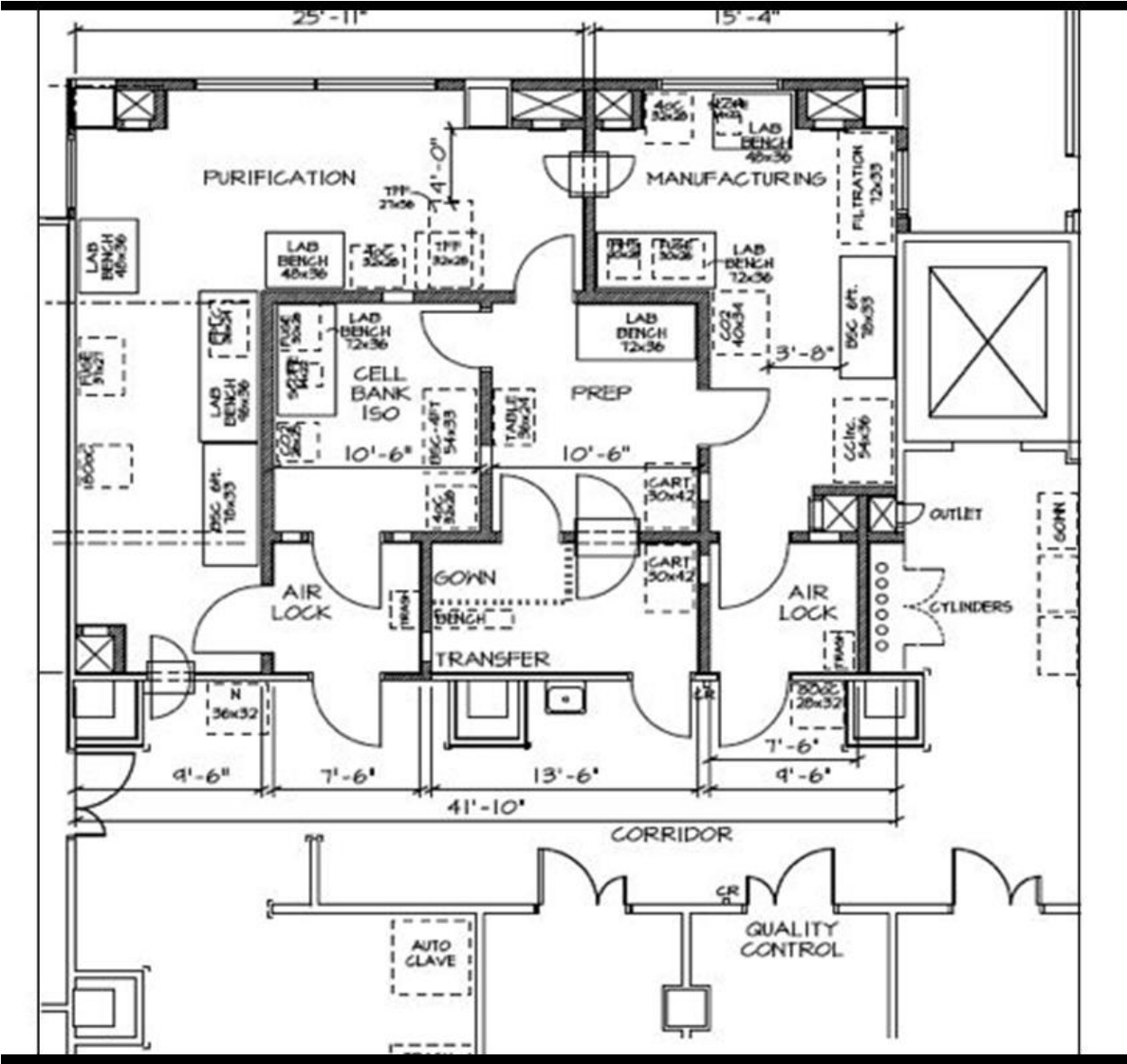


Figure 14.1: Sample facility design.

## 15. Conclusions and Recommendations

Ultimately, it is recommended that the fluidized bioreactor process be implemented for the new facility. However, before the process is implemented, pilot-scale studies should be conducted to verify that the calculations and assumptions made in the project are applicable on a larger scale. Assuming an 85% yield for the overall process and capturing 70% of the domestic and European markets, the overall production goal is 650 kg of rituximab. The fluidized bioreactor process requires only one process running for 212 days per year while the traditional stirred tank process requires 4 parallel processes running for 325 days to reach the production goal. Furthermore, the overall product yield per day for the fluidized bioreactor process is about 7-fold greater. An incremental analysis between the two processes at an assumed MARR of 18% gives a differential present worth of about \$13 billion indicating that it is a favorable project. The fluidized bioreactor process has a higher total permanent investment but ultimately offers a more profitable payout.

The fluidized bioreactor process is also superior to the stirred tank process for significant changes in plant life, variable cost, overall product yield, product price, and cost of capital. It is an extremely innovative process that requires deviation from the traditional stirred tank structure, but ultimately offers the solution to a key industry problem. It allows cells to grow to a higher cell density by two orders of magnitude, which ultimately allows for increased product formation. Despite disadvantages including increased media requirements and a heavier load on downstream purification, the fluidized bioreactor process is superior to the traditional stirred tank process.

## 16. Acknowledgements

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## Appendix A: Minimum Fluidization Velocity Calculations

Particle Volume Calculation

$$V = 4\pi abc$$

Where a = major axis radius, b = minor axis radius, and c = vertical axis radius.

Particle Surface Area Calculation

$$SA = 4\pi \left( \frac{(ab)^{1.6} + (ac)^{1.6} + (bc)^{1.6}}{3} \right)^{1/1.6}$$

Effective Spherical Diameter

$$D = \frac{6 * V}{SA}$$

Three values were calculated for D;  $D_{\max} = 1.31\text{E-}03$  m,  $D_{\text{avg}} = 9.12\text{E-}04$  m, and  $D_{\min} = 4.98\text{E-}04$  m.

Minimum fluidization velocities were calculated for each of the three diameter sizes using the Ergun equation coupled with the bed mass balance:

$$g(\rho_p - \rho_f) = \frac{150\mu_f (1 - \varepsilon_M)}{\Phi_s^2 D_p^2 \varepsilon_M^3} V_{oM} + \frac{1.75\rho_f}{\Phi_s^2 D_p} \frac{1}{\varepsilon_M^3} V_{oM}^2$$

Values

$\rho_s = 1320 \text{ kg/m}^3$ ;  $\rho_f = 992.9 \text{ kg/m}^3$ ;  $\mu_f = 6.73\text{E-}4 \text{ kgm}^{-1}\text{s}^{-1}$ ;  $\Phi_s = 0.99$ ;  $\varepsilon_M = 0.35$ .

Solving for

$D_{\max}, V_{oM} = 0.003158 \text{ m/s}$

$D_{\text{avg}}, V_{oM} = 0.001643 \text{ m/s}$

$D_{\min}, V_{oM} = 0.000507 \text{ m/s}$

## Appendix B: Nutrient Transport in a Fluidized Bed Reactor

Cell-liquid mass transport. For Glucose, in microcarrier culture

$[S]_b$  defined as the concentration of species S, glucose, in the bulk

$[S]_s$  defined as the concentration of species S, glucose, at the surface of the cell

Y defined as the cell surface coverage on the microcarrier

$R_s$  is defined as the uptake rate per cell of species S.

$Sh_s$  is the Sherwood Number, given by the relation below.

$D_s$  is the diffusion coefficient for glucose in media at 37°C.

$$Sh_s = 2 + 0.552 Re^{\frac{1}{2}} Sc_A^{1/3}$$

Where  $Sc$  is the Schmidt Number which is the ratio of the viscous diffusion rate to the molecular mass diffusion rate.

$$Sc_s = \frac{\mu_f}{\rho_f D_s}$$

And  $Re$  is the Reynold's Number, given by the correlation for flow in an annular channel

$$Re = \frac{V(D_o - D_i)\rho_f}{\mu_f}$$

The Sherwood number for the packed bed bioreactor,  $Sh_{s,pb} = 16$ ;

The Sherwood number for the fluidized bioreactor,  $Sh_{s,fb} = 338.3$ .

The normalized concentration gradient of Glucose between the bulk medium and at the cell surface is given by the expression:

$$\frac{[S]_b - [S]_s}{[S]_b} = \frac{Y R_s d_e}{D_A [S]_b Sh_s}$$

Using values of  $Y = 3.53E+08 \text{ cell/m}^2$ ;  $R_s = 4.03E-02 \text{ mol cell}^{-1}\text{day}^{-1}$ ;  $D_s = 0.0000864 \text{ m}^2\text{day}^{-1}$ ;  $d_e = 0.000911795 \text{ m}$ ;  $[S]_b = 44.406 \text{ mol/m}^3$ ; and the Sherwood Relations given above,

$$\frac{[S]_b - [S]_s}{[S]_b} = 0.01 \text{ for the fluidized bed bioreactor}$$

$$\frac{[S]_b - [S]_s}{[S]_b} = 0.27 \text{ for the packed bed bioreactor}$$

## Appendix C: Fluidized Bed versus Packed Bed—Nutrient Composition Temporal Analysis

Assumptions:

- 1) Media has 8 g/L of glucose initially and the reactor has been charged initially with media.
- 2) Cells in exponential growth phase ( $\mu_{net} = \mu_g$ )
- 3) No notable cell loss from the reactor, nor notable gain from the incoming stream.
- 4) Glucose consumption is solely due to metabolic consumption of glucose by the cell. No glucose consumption is attributed directly to product formation.
- 5) All reactor systems are well mixed, such that there exists no spatial dependence on cell or substrate concentrations inside the reactor at a given time point.

Cell Growth

X is defined as cell concentration within the reactor

$\mu_{net}$  is defined as the specific growth rate of the cell culture

$$\frac{dX}{dt} = \mu_{net}X$$

Solving for  $\mu_{net}$ :

$$\ln\left(\frac{X}{X_o}\right) = \mu_{net}t$$

$$t = 12.8 \text{ days}; \mu_{net} = 0.3325 \text{ day}^{-1}$$

Substrate Consumption

Glucose mass balance is given by the equation below:

$$V_R \frac{dS}{dt} = F(S_o - S(t)) - \frac{V_R \mu_{net}}{Y_{X/S}} X(t)$$

Where

$V_R$  is the Volume of the Reactor (L);

F is the flow rate of the nutrient rich stream through the reactor (L/day);

$S_o$  is the concentration of glucose at time zero (g Glucose/L);

S is concentration of glucose in the reactor at time t (g Glucose/L);

$\mu_{net}$  is the specific growth rate calculated above ( $\text{day}^{-1}$ );

$Y_{X/S}$  is the yield coefficient of cells on glucose (g cell/g glucose);

X(t) is the concentration of cells in the reactor at time t (g cell/L).

This equation was solved as follows:

$$\begin{aligned} V_R \frac{dS}{dt} &= F(S_o - S(t)) - \frac{V_R \mu_{net}}{Y_{X/S}} X(t) \\ \frac{dS}{dt} &= \frac{F}{V_R} (S_o - S(t)) - \frac{\mu_{net}}{Y_{X/S}} X(t) \\ \frac{dS}{dt} + \frac{F}{V_R} S &= \frac{F}{V_R} S_o - \frac{\mu_{net}}{Y_{X/S}} X(t) \end{aligned}$$

$$\begin{aligned}
L\left(\frac{dS}{dt} + \frac{F}{V_R} S\right) &= L\left(\frac{F}{V_R} S_o - \frac{\mu_{net}}{Y_{X/S}} X(t)\right) \\
sS(s) + \frac{F}{V_R} S(s) - S_o &= \frac{F}{V_R} \frac{S_o}{s} - \frac{\mu_{net}}{Y_{X/S}} X(s) \\
S(s) \left(s + \frac{F}{V_R}\right) &= \left(\frac{F}{V_R} + s\right) \frac{S_o}{s} - \frac{\mu_{net}}{Y_{X/S}} X(s) \\
S(s) &= \frac{S_o}{s} - \frac{\frac{\mu_{net}}{Y_{X/S}}}{\left(s + \frac{F}{V_R}\right)} X(s) \\
X(s) &= \frac{X_o}{(s - \mu_{net})} \\
S(s) &= \frac{S_o}{s} - \frac{\frac{X_o \mu_{net}}{Y_{X/S}}}{\left(s + \frac{F}{V_R}\right)(s - \mu_{net})} \\
S(s) &= \frac{S_o}{s} + \frac{\frac{\frac{X_o \mu_{net}}{Y_{X/S}}}{\left(\frac{F}{V_R} + \mu_{net}\right)}}{\left(s + \frac{F}{V_R}\right)} - \frac{\frac{\frac{X_o \mu_{net}}{Y_{X/S}}}{\left(\frac{F}{V_R} + \mu_{net}\right)}}{(s - \mu_{net})}
\end{aligned}$$

Which leads to the final result that:

$$S(t) = S_o + \frac{X_o \mu_{net}}{Y_{X/S} \left(\frac{F}{V_R} + \mu_{net}\right)} e^{-\frac{F}{V_R} t} - \frac{X_o \mu_{net}}{Y_{X/S} \left(\frac{F}{V_R} + \mu_{net}\right)} e^{\mu_{net} t}$$

With values of  $Y_{X/S} = 0.411$  g cell/g glucose;  $X_o = 1.9$  g cell/L;  $V_R = 20000$  L;  
 $\mu_{net} = 0.3325$  day<sup>-1</sup>;  
 $S_o = 8$  g/L.

For Batch reactor,  $F = 0$  L/day; for the Packed Bed Bioreactor,  $F = 106262.2$  L/day; for the Fluidized Bioreactor,  $F = 671152$  L/day. These values were substituted into the equation and plotted for each bioreactor confirmation over the entire growth time of 12.8 days. The results are plotted below:

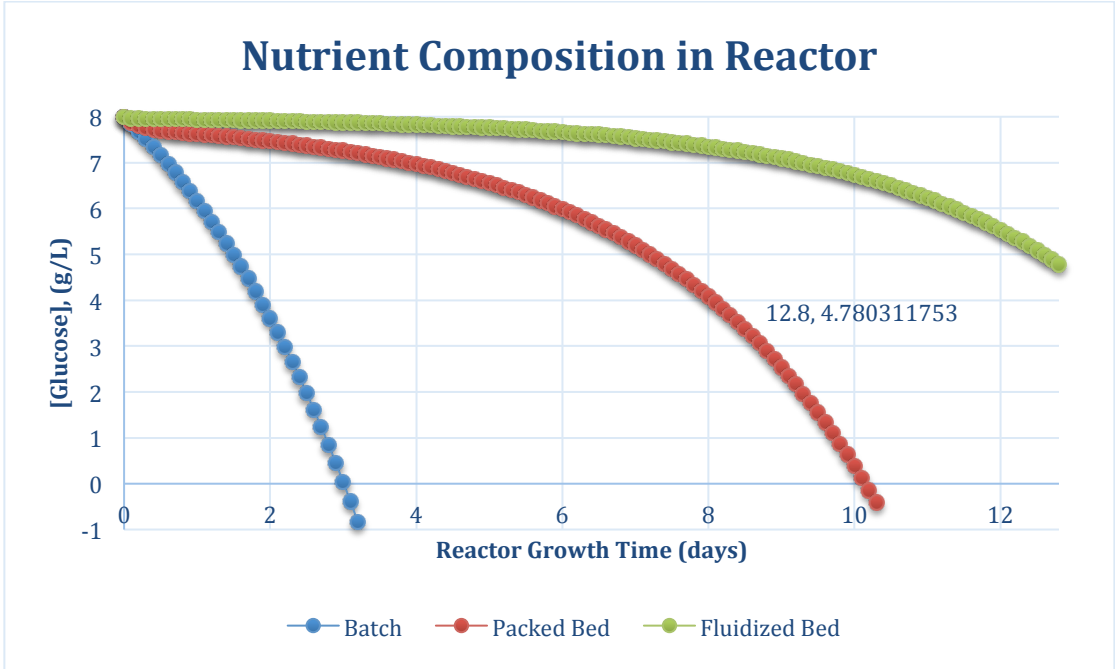


Figure 1: The Glucose concentration plotted over the total growth time in different reactor confirmations. The blue line corresponds to batch operation, the red line corresponds to packed bed operation, and the green line corresponds to a fluidized bioreactor operation. In the packed bed and batch operation, the reactor will become nutrient deprived (<4g/L of glucose) before the end of the growth period. The fluidized bed bioreactor will be able to sustain cell growth and culture throughout the entirety of the growth period, having 4.78 g/L of glucose by the end of the 12.8 day growth period.

## Appendix D: Fluidized Bed versus Packed Bed—Nutrient Composition Spatial Analysis

Assumptions:

- 1) Media has 8 g/L of glucose initially and the reactor has been charged initially with media.
- 2) Cells in exponential growth phase ( $\mu_{\text{net}} = \mu_{\text{g}}$ )
- 3) No notable cell loss from the reactor, nor notable gain from the incoming stream.
- 4) Glucose consumption is solely due to metabolic consumption of glucose by the cell. No glucose consumption is attributed directly to product formation.

With spatial gradients, the diffusion equation becomes

$$\frac{\partial S}{\partial t} + \nabla(-D_S \nabla S) + u \nabla S = R_i$$

Where

$$R_i = \frac{\mu_{\text{net}}}{Y_{\frac{X}{S}}} X(t)$$

$u = 0.0005 \text{ m/s}$  in the + y direction;

$D_S = D_S = 0.0000864 \text{ m}^2 \text{ day}^{-1}$

These numbers were programmed into the Comsol Multiphysics 2D Transport of Diluted Species module. The results are seen in Figure 2 below.



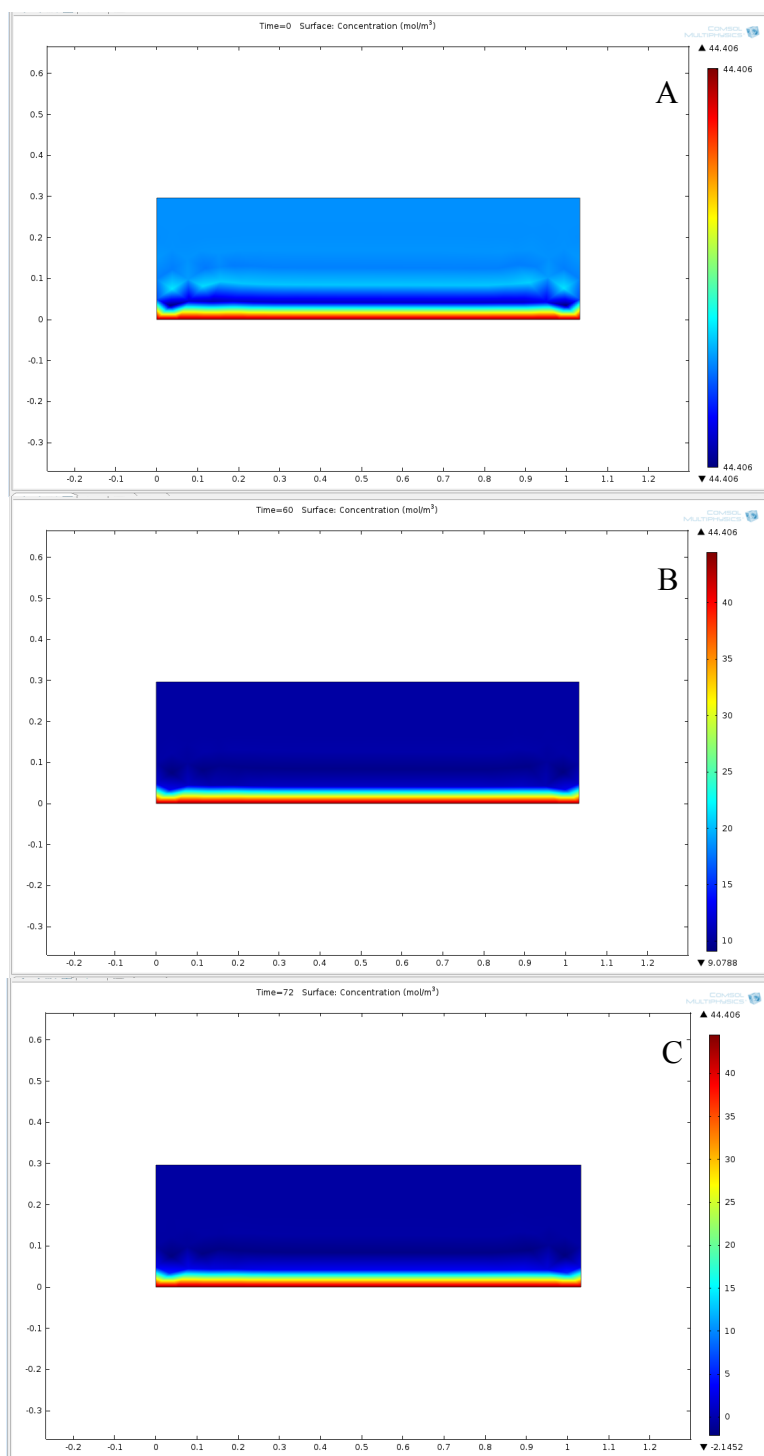


Figure D1: Comsol Multiphysics surface plots of Glucose Concentration Gradients in a Packed Bed Reactor. Surface concentration gradients are shown when the reactor was initially charged (A), after running for 60 hours (B), and after running for 72 hours (c). Initially, the concentration is uniform at 44.406  $\text{mol/m}^3$  in A. By 60 hours (B), the majority of the packed bed will only have 9.27  $\text{mol/m}^3$  (1.93 g/L) of glucose. After 72 hours, the majority of the bed will have 0 g/L of glucose. The reactor will be nutrient deprived after 60 hours, and have no glucose propagating through by 72 hours.

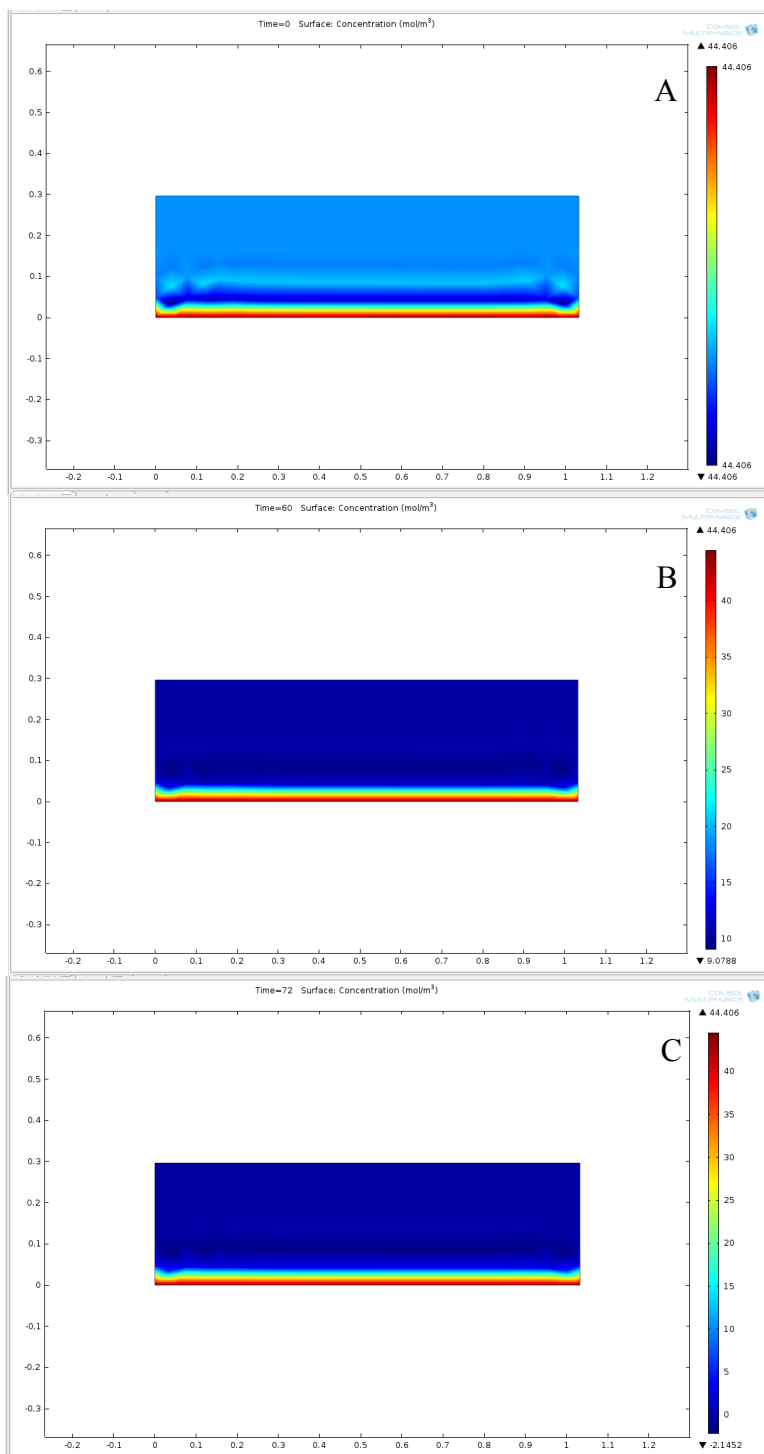


Figure D2: Comsol Multiphysics surface plots of Glucose Concentration Gradients in a Packed Bed Reactor. Surface concentration gradients are shown when the reactor was initially charged (A), after running for 60 hours (B), and after running for 72 hours (c). Initially, the concentration is uniform at  $44.406 \text{ mol/m}^3$  in A. By 60 hours (B), the majority of the packed bed will only have  $9.27 \text{ mol/m}^3$  ( $1.93 \text{ g/L}$ ) of glucose. After 72 hours, the majority of the bed will have  $0 \text{ g/L}$  of glucose. The reactor will be nutrient deprived after 60 hours, and have no glucose propagating through by 72 hours.

Appendix E: Design Calculations for Fluidized Bioreactor

Fluidized Bed Calculations			Goal Seek		TRUE	Cytoline 1 Max specs		
Cytoline 1 Avg Specs	#VALUE!	Units	2.06633E-08			Density	#VALUE!	Units
Density	1320	kg/m^3			Length	1.25E-03	m	
Length	1.05E-03	m			Thickness	5.50E-04	m	
Thickness	3.75E-04	m			Vol	1.58E-09	m^3	
Vol	6.19E-10	m^3			SA	7.26E-06	m^2	
SA	4.07E-06	m^2			Effective D	1.31E-03	m	
Effective D	9.12E-04	m			Sphericity	0.99		
Sphericity	0.99				Min Fluidization	0.003158	m/s	
Bed Specs	#Value!	Units						
Operating Temp	37	C						
Fluid Viscosity	6.73E-04	kg/(m*s)						
Media Density	992.9	kg/m^3						
Void Fraction	0.35							
Min Fluidization	0.001643	m/s						
Actual Velocity	0.003158	m/s						
Fluidized Bed Operating Specs			1.922404		Multiple of the average fluidization velocity			
			1		Multiple of the max fluidization velocity			
Re	6.53E+00		6.23347		Multiple of the minimum fluidization velocity			

Fluidized Bed Sizing of the Reactor and Pressure Drop Sheet

Working Vol	25000 L	Working Velocity	Fluid Mechanics through an Annulus	
	25 m^3	Area of Annulus	0.003158 m/s	
"Aspect Ratio"	h:3D	Wetted Perimeter	2.459773 m^2	
"Annular" Diameter	1 m	Effective Diameter	9.527516 m	
Actual D of Inside Cavity	0.9492 m		1.032703 m	
Goal Seek	-1.6E-05	Flow rate	0.007768 m^3/s	
Diameter FB	2.032703 m	Volumetric	466.0778 L/min	
Height	8.130811 m		27964.67 L/hr	
Working Height	8.130832	Fluid Viscosity	6.73E-04 kg/(m*s)	
Annular Ac	2.459773 m^2	Media Density	992.9 kg/m^3	
Annular Vol	19.99995 m^3	Re	4.81E+03	
Inner Ac	0.707629 m^2	Mass Flow Rate	7.712811 kg/s	
Volume of Inner	5.753594 m^3			
alpha	0.28768			
Cross sectional area	3.245171 m^2	e/d outer	7.38E-06	0.002
Total Vol of Reactor	26.38587 m^3	e/d inner	1.50E-05	0.0025
Bed Calculations		Pressure Drop Across Non Bed Annulus	-76306.7 Pa	
Volume of Bed	0.72993 m^3	Pressure Drop Across Non Bed Annulus	-79197.1 Pa	
Height of Bed (pre)	0.296747 m	Pressure Drop Down Recirculation Channel	-79116.4 Pa	
Height of Bed (flu)	0.741867 m			
Volume of Flu Bed	1.824825 m^3			

Cell Growth Profile	
Starting Cell Number	1.45985E+12 Cells
Ending Cell Number	1.0219E+14 Cells
Growth Time	12.77777778 day
mu_net	0.332490932 day <sup>-1</sup>
mu_net	1.39E-02 hr <sup>-1</sup>
mu_net/PaperMu	3.15E+00

Glucose Uptake Paper	10.08 mmol/10 <sup>6</sup> cell/day
Glucose Uptake	4.03E-02 mol/10 <sup>6</sup> cell/day
	7.26E+00 g glucose/10 <sup>6</sup> cell/day
	9.28E+01 g glucose/10 <sup>6</sup> cell
	2.43E+00 g glucose/g cell
Yx/s	4.11E-01 g cell/g glucose

Glucose Concentration	
[S_o]	8 g/L
Glucose Initially in Reactor	160000 g glucose

Batch Reactor	
Growth Time	3.581521543 Days
Ending Concentration	-6.81E-05 g/L
Amount of Cells in Reactor	3.97E+12 Cells

Equivalent Spherical Diameter	0.000911795 m
Chemical surface coverage	3.53E+08 cells/m <sup>2</sup>
Uptake Rate	4.03E-02 mol/cell/day
Diffusivity of Glucose	1000 um <sup>2</sup> /s
	0.000000001 m <sup>2</sup> /s
	0.0000864 m <sup>2</sup> /day
Schmidt Number	678.0743277
Reynolds number through bed	4809.613077
Sherwood number	338.3204289
Bulk difference	0.010007182

### Assumptions

- 1) Cells in exponential growth phase
- 2) No notable cell loss/gain into the reactor
- 3) 1 cell =  $2.62 \times 10^{-11}$  kg
- 4)  $\mu_g = \mu_{net} \Rightarrow$  cells in exponential growth phase
- 5) Assume glucose consumption is solely due to metabolic consumption of the cell and not due to product formation

1 cell	2.62E-11 kg
10^6 cell	2.62E-05 kg
	2.62E-02 g
1 cell	2.62E-08 g

Diffusion coefficient

of Glucose in Water at 37C

1000  $\mu\text{m}^2/\text{sec}$

### Volume of Bed

0.72993

### Initial Cell Concentration

3.67E+06 g/m<sup>3</sup> $Y_{x/s}$ 

0.411 gcell/gglucose

MW glucose

180.1559 gglucose/mol

 $Y_{x/s}$ 

74.0440749 gcell/molglucose

mu net

0.0139 hr<sup>-1</sup>

So

44.4059839 mol/m<sup>3</sup>

### Viscosity of media

6.73E-04 kg/(m\*s)

### Density of media

992.9 kg/m<sup>3</sup>

### Volume of 1 Microcarrier

6.185E-10 m<sup>3</sup>

Number of Microcarriers

1178656056 microcarriers

### Surface Area of Cytoline 1

 $0.3 \text{ m}^2/\text{g}$ 

### Volume of Microcarriers

7.29E-01 m<sup>3</sup>

### Density of Microcarriers

1320 kg/m<sup>3</sup>

g microcarriers

9.62E+05 g

SA

2.89E+05 m^2

Cells/SA

3.53E+08 cells/m<sup>2</sup>

## Appendix F: Process Design Calculations

### Stirred Tank Process

Innoculum	
Volume (L)	2
Cell Concentration (cells/mL)	1.00E+07

First Cultivator CSTR	
Working Volume (L)	100
Concentration after Diluting (cells/mL)	2.00E+05
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	2.30E+05
Target Cell Concentration (end of batch) (cells/mL)	2.00E+06
Cell Growth Time (day)	4
SIP+CIP>Loading Time (day)	0
Total Time (day)	4
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	6.61E+05
Product Formation (g)	23.7888
Final Cell concentration (cells/ml)	1.12E+06

Second Cultivator CSTR	
Working Volume (L)	1000
Concentration after Diluting (cells/mL)	1.12E+05
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	129208.32
Target Cell Concentration (end of batch) (cells/mL)	3.00E+06
Cell Growth Time (day)	4
SIP+CIP>Loading Time (day)	0
Total Time (day)	4
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	3.71E+05
Product Formation (g)	133.4075904
Final Cell Concentration (cells/ml)	6.29E+05

Third Cultivator CSTR	
Working Volume (L)	20000
Concentration after Diluting (cells/mL)	3.14E+04
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	3.62E+04
Target Cell Concentration (end of batch) (cells/mL)	3000000
Cell Growth Time (day)	16
SIP+CIP>Loading Time (day)	0.166666667
Total Time (day)	16.16666667
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	3.21E+05
Product Formation (g)	9253.145302
Final Cell concentration (cells/ml)	6.11E+05

Three CSTR Process	
Final Product/Batch (kg)	9.410
Total Batch Time (days)	24.167
Number of Batches/Year	19.000000
Product/Year (kg)	178.7964922
Processes to meet production goal	4.284200382

### Fluidized Bed

Inoculum Prep	
Working Volume (L)	0.1
Initial Cell Concentration (cells/mL)	2.00E+06
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	2.30E+06
Target Cell Concentration (end of batch) (cells/mL)	1.00E+07
Cell Growth Time (day)	3.47E+00
Final Cell Concentration (cells/ml)	1.00E+07
30 Bag Bioreactors for total of 3L inoculum	



First Cultivator CSTR	
Working Volume (L)	33.33333333
Concentration after Diluting (cells/mL)	3.00E+05
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	345600
Target Cell Concentration (end of batch) (cells/mL)	2000000
Cell Growth Time (day)	4.918981481
SIP+CIP>Loading Time (day)	0
Total Time (day)	4.918981481
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	1150000
Product Formation (g)	16.97048611
Final Cell Concentration (cells/ml)	2.00E+06

First Cultivator CSTR	
Working Volume (L)	33.333333
Concentration after Diluting (cells/mL)	3.00E+05
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	345600
Target Cell Concentration (end of batch) (cells/mL)	2000000
Cell Growth Time (day)	4.918981
SIP+CIP>Loading Time (day)	0
Total Time (day)	4.918981
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	1150000
Product Formation (g)	16.97049
Final Cell Concentration (cells/ml)	2.00E+06

First Cultivator CSTR	
Working Volume (L)	33.33333333
Concentration after Diluting (cells/mL)	3.00E+05
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	345600
Target Cell Concentration (end of batch) (cells/mL)	2000000
Cell Growth Time (day)	4.91898148
SIP+CIP>Loading Time (day)	0
Total Time (day)	4.91898148
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	1150000
Product Formation (g)	16.9704861
Final Cell Concentration (cells/ml)	2.00E+06

Second Cultivator CSTR	
Working Volume (L)	1000
Concentration after Diluting (cells/mL)	200000
Specific Growth Rate (1/day)	1.152
Growth Rate (cells/mL*day)	230400
Target Cell Concentration (end of batch) (cells/mL)	1459854
Cell Growth Time (day)	5.468116
SIP+CIP>Loading Time (day)	0
Total Time (day)	5.468116
Product Growth Rate (pg/cell*day)	90
Average Cell Density (cells/mL)	829927
Product Formation (g)	408.4324
Final Cell Concentration (cells/ml)	1459854

Fluidized Bed	
Working Volume (L)	20000
g microcarriers/L media	50
Density of Microcarriers (g/mL)	1.32
L microcarriers/L media	0.037878788
Volume of Media (L)	19270.07299
Volume of Microcarriers (L)	729.9270073
Initial Cell Density (cells/mL microcarrier)	2.00E+06
Initial Number of Cells	1.45985E+12
Target Cell Density (cells/mL microcarrier)	1.40E+08
Target Number of Cells	1.02E+14
Cell Productivity (g product/L microcarrier-day)	1
Product Formation/day (g)	729.9270073
Growth Rate (cell/mL carrier*day)	1.08E+07
Product formation (pg/cell*day)	50
Cell Growth Time (day)	12.77777778
Cleaning Time + Loading Time (day)	0.208333333
Total Time (day)	12.98611111
Product Formation (g)	65287.91565

Final Product/Batch (kg)	65.747
Total Batch Time (days)	23.373
Number of Batches/Year	12.000000
Product/Year (kg)	788.9671
Processes to meet production goal	0.97089

## Appendix G: CIP and SIP Procedures

Clean-in-place and steam in place (CIP and SIP) procedures are used in biological processes to sterilize permanent equipment between each batch. These procedures are critical to preventing cross contamination<sup>2</sup>.

The following CIP procedure will be used for the process where required:

Each vessel will be washed with an amount of sterile water equal to half its volume for 10 minutes.

Next, a cleaning solution of caustic (NaOH 0.5 M) equal to half the vessel's volume will be sprayed into the tank.

Sterile water will then be used as in step 1 to wash away the cleaning solution for 10 minutes.

Next, a cleaning solution of acid (H<sub>3</sub>PO<sub>4</sub> 5% w/w) equal to half the vessel's volume will be sprayed into the tank.

Sterile water will then be used as in step 1 to wash away the cleaning solution.

Lastly, another half volume of sterile water will be used to wash the vessel once more before the next batch. This washing regime will use approximately 2 vessel volumes of water. This includes the water to be used to make the sterile wash solution.

For certain units, the CIP procedure may be determined by the manufacturer protocol.

---

Steam-in-place procedures are required for large, sturdy pieces of equipment such as stainless steel bioreactors.

The following SIP procedure will be used for the process where required:

Each reactor will be washed with an amount of sterile water equal to half its volume.

Next, a cleaning solution of caustic (NaOH 0.5 M) equal to half the vessel's volume will be sprayed into the vessel.

Sterile water will then be used as in step 1 to wash away the cleaning solution.

---

<sup>2</sup> Stewart, J.m Seiberling, D., "The Secret's Out: Clean in Place" Chemical Engineering, 1996

Next, a cleaning solution of acid ( $\text{H}_3\text{PO}_4$  5% w/w) equal to half the vessel's volume will be sprayed into the tank.

Sterile water will then be used as in step 1 to wash away the cleaning solution.

Five lb/hr of pure steam per cubic foot of vessel volume will be pumped into the vessel to heat it up to the temperature of the steam, 130°C. Note: The steam used in this procedure will be generated by a sterile steam generator at 130°C and 2 bars.

Once the 130°C has been reached in the vessel, the steam flow rate will be reduced to one lb/hr of pure steam per cubic foot of vessel volume and held at the flow rate.

Lastly, after thirty minutes, the flow rate of steam will be stopped and a half volume of sterile water will then be sprayed into the tank to wash it and cool it down to room temperature.

For certain units, the SIP procedure may be determined by the manufacturer protocol.

**Appendix H: Vendor Sheets**

# Cytopilot Mini reactor – low volume, high productivity

**M**aterial for the production of biopharmaceuticals for therapeutics and diagnostics is often produced using different cell culture techniques. One technique is to use microcarriers for cell culture.

Microcarriers help improve yield, lower serum and media costs and provide a low physical and chemical stress environment for anchorage dependent cells.

Cytopilot™ Mini is a laboratory-scale reactor that achieves high productivity from a small reactor volume. It is designed for maximum ease of use, with easy filling and discharging, simple cleaning-in-place (CIP) routines and reliable operation. Cytopilot Mini achieves very high cell densities of immobilised cells which, due to its novel method of oxygen and nutrient transfer, it can sustain in a continuous perfusion production system. Such high cell densities often require volumes of culture medium per day in the order of 25 times the bed matrix volume, depending on the optimisation of culture.

## High productivity

The reactor is easy to use and highly productive. As an indication of its productivity, Cytopilot Mini together with Cytoline™ microcarriers can give a volumetric productivity of 400 mg antibodies/litre microcarriers per day, with a titre of up to 100–200 mg/l (data supplied by customer) from a hybridoma culture. Furthermore, processes run on Cytopilot Mini are easy to scale up, reactors at 100 litre scale are already in operation.



Figure 1. Cytopilot Mini fluidised bed reactor has a total volume of 2 litres with a working volume of 100–500 ml microcarriers. It has a novel internal circulation loop through which oxygen microbubbles circulate, ensuring a high level of dissolved oxygen throughout the fluidised bed.

**Cytopilot Mini is a laboratory-scale reactor which enables cell culture on porous Cytoline microcarriers in a low shear stress environment. Cytopilot Mini provides controlled, fluidised bed, perfusion culture for the evaluation of parameters for engineering and feasibility studies, for product testing, or as a production tool for diagnostic purposes. Cytopilot Mini is now available world-wide.**

## Easy change to serum-free media

Continuous supply of nutrients to cells in a fluidised bed constitutes the perfusion culture. A perfusion culture system like Cytopilot Mini gives very stable culture conditions and facilitates changing from serum-containing to serum-free media, or switching from growth to production media.

## How does it work?

Figure 2 describes the principle of operation. Cytopilot Mini consists of two chambers separated by the distribution plate. The novel internal recycling loop runs through both chambers. Liquid agitated by the magnetic stirrer in the bottom chamber is conveyed via the distribution plate into the upper chamber. Settled microcarriers are then lifted by hydrodynamic pressure to form a fluidised bed. The degree of expansion of the fluidised bed is dependent on the stirrer speed. When the bed is fluidised, a clear zone forms between the top of the suspended microcarriers and the top of the culture medium. Culture medium circulates back in through the upper sieve/filter and down through the internal recycling loop to the stirrer in the lower chamber.

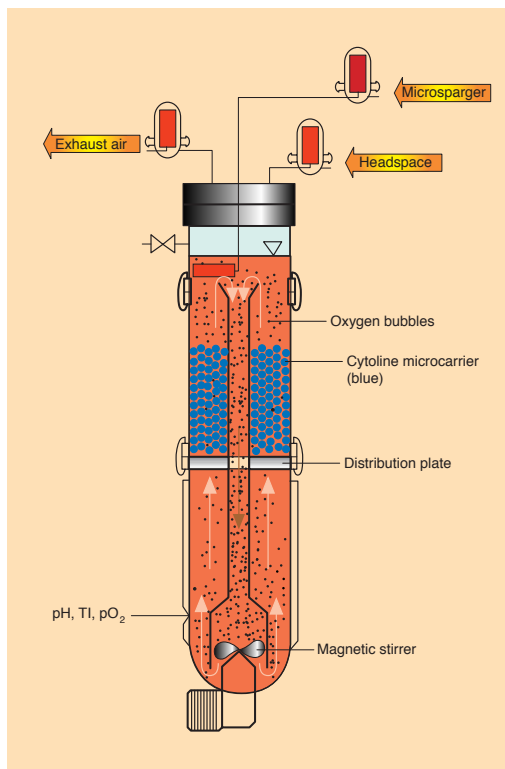


Figure 2. Principle of oxygenation in Cytopilot fluidised bed reactors. Oxygen microbubbles are sparged into the culture medium as it is drawn down through the central recycling loop.

## Near-perfect oxygen transfer

Oxygen bubbles are sparged into the culture medium in the upper microcarrier-free zone. Coalescent bubbles immediately rise to the surface and only the liquid saturated with microbubbles flows down through the recycling loop to the magnetic stirrer. The gas bearing capacity of these microbubbles provides the system with an oxygen reserve. The microcarriers are freely suspended in the medium, and statistically, all microcarriers obtain the same amount of oxygen and nutrients. This near-perfect oxygen transfer minimises the formation of gradients in the culture medium and makes scale up very simple. Furthermore, excess CO<sub>2</sub> can be drawn off via the headspace. The benefit gained from this type of aeration system and cells immobilised inside the porous matrix of Cytoline, is the dramatic reduction in cell damage.

## Cytoline, its favoured partner

Optimal fluidisation of the microcarriers is achieved by selecting the microcarrier with optimum features, i.e. specific density, size, rigidity, non-abrasive, simple to use, sterilisable. Cytopilot Mini fluidised bed reactor is designed

specifically to exploit the potential of Cytoline microcarriers. These are macroporous microcarriers weighted with silica designed for culture of CHO cells, hybridoma and other stress sensitive cells. Cytopilot Mini together with Cytoline provides a controlled fluidised bed, perfusion environment.

## Cell cultures and areas of use

Cytopilot Mini is useful for evaluating fluidised bed cell culture at laboratory-scale, feasibility studies for large-scale cell culture and for producing enough material for product testing or diagnostic purposes. It is ideal for use with the following cell cultures:

- Adherent cell lines with strong anchorage properties, for example fibroblast cells in combination with heavy microcarriers such as Cytoline 1 with a high sedimentation rate.
- Semi-adherent cells in combination with heavy or light microcarriers such as Cytoline 1 and 2, respectively.
- Suspension cell lines in combination with light macroporous carriers (Cytoline 2) for very sensitive cells or heavy microcarriers (Cytoline 1) for more rigid cells.

### *For more information, ask for the following Data Files:*

Cytopilot Mini	Code no. 18-1060-74
Cytoline 1 and Cytoline 2 macroporous microcarriers	Code no. 18-1060-65

### *Other microcarrier products:*

Cytodex™ microcarriers for cell culture	Code no. 18-1060-61
Cytopore™ macroporous microcarriers	Code no. 18-1132-68





## **Thermo Scientific Precision Incubators**

*Performance, Quality and Value for Your Incubation Needs*



# Global Leader in **Quality and Reliability**

For more than 50 years, Thermo Scientific products have been trusted by the world's leading biotechnology, pharmaceutical, academic, industrial and clinical laboratories. Our solutions deliver the performance, quality and reliability required by researchers and clinicians worldwide.



# Thermo Scientific Precision Incubators



Our new Thermo Scientific Precision incubator series delivers excellent temperature uniformity, performance and value for a wide range of microbiological incubation applications – from everyday needs to demanding incubation and storage tasks.

## **Precision® High-Performance Incubators** pg. 3-6

- > Superior Temperature Uniformity

## **Precision Standard Incubators** pg. 7

- > For Everyday Incubation Applications

## **Precision Compact Incubators** pg. 8

- > Space-Saving Design for Basic Applications

## **Precision Refrigerated Incubators** pg. 9-10

- > For Various Applications at Temperatures Below and Above Ambient

APPLICATIONS	Precision High-Performance Incubators	Precision Standard Incubators	Precision Compact Incubators	Precision Refrigerated Incubators
Bacterial Research		•	•	•
Biochemical Oxygen Demand (BOD)				•
Chemical Storage	•	•		•
Chick Embryo Studies		•	•	
Coliform Determinations	•	•	•	•
Crystallization Studies	•	•		
Drying And Staining Procedures	•	•		•
Entomological Studies				•
Enzyme Digestion Studies	•	•		•
Hematological Testing	•	•	•	•
Microbiological Determinations	•	•	•	•
Paraffin Embedding	•	•		
Pharmaceutical Stability Testing	•			
Sample Storage	•			•
Tissue Culturing	•	•	•	•
Plant Growth Studies				•

# Thermo Scientific Precision High-Performance Incubators



## Mechanical or Gravity Convection

Featuring advanced microprocessor controls, our Precision high-performance incubators are available with mechanical or gravity convection for your most important applications that require excellent temperature distribution or gentle sample incubation and handling between 5°C above ambient to 75°C.



### Choice of Mechanical or Gravity Convection

- Mechanical convection provides uniform heating and precise temperature control. A blower circulates heated air in a horizontal airflow pattern for efficient heat distribution with tight temperature tolerances of up to  $\pm 0.3^{\circ}\text{C}$
- Gravity convection offers safe incubation with reduced air changes, minimizing drying-out of samples while providing a stable environment

### Advanced Microprocessor Controls

- Sophisticated microprocessor controls feature easy-to-view digital LED readout and reliable temperature control
- Fixed setpoints on control panel eliminate the need for tuning and off-set feature enables easy calibration
- Temperature is displayed on large, three-character screen and can be easily set from 5°C above ambient to 75°C in 0.1°C increments using touch-sensitive arrow keys

### Built-In Safety

- Built-in safety back-up maintains control at 3°C above setpoint if primary heater control fails
- Visual alarm indicates when temperature exceeds 3°C setpoint
- Silicone gasket on outer door and 3" thick fiberglass insulation prevents heat loss and ensures excellent temperature uniformity
- Circuit breaker protects against power surges.

### Simplicity and Flexibility

- Inner glass door permits viewing of samples without disturbing the chamber environment
- Internal electrical outlet allows operation of a shaker, stirrer or other lab apparatus

### Durable and Robust

- Cabinet has durable, enamel-coated steel exterior. Interior chamber features easy-to-clean stainless steel
- Low-watt density heater elements are designed for long life and energy efficiency

### Mechanical Convection Models

- Stackable, table-top units
- Outer door opens to 180° for unhindered access
- Double door unit and large capacity units also available
- Choice of 120V and 240V versions
- Internal electrical outlet in all models



High-Performance Mechanical Convection				Double Doors	Large Capacity
<b>Chamber Volume</b>	2.2 cu. ft./62 L	3.4 cu. ft./96 L	4.5 cu. ft./127 L	11.2 cu. ft./317 L	30 cu. ft./850 L
<b>Temperature Control</b>	Microprocessor PID	Microprocessor PID	Microprocessor PID	Hydraulic Thermostat	Hydraulic Thermostat
<b>Temperature Display</b>	LED	LED	LED	LED	Thermometer
<b>Temperature Range</b>	ambient +5° to 75°C	ambient +5° to 75°C	ambient +5° to 75°C	ambient +5° to 65°C	ambient +5° to 70°C
<b>Uniformity @37°C</b>	±0.5°C	±0.5°C	±0.5°C	± 0.6°C	±0.5°C
<b>Dimensions (W x H x D)</b>					
Chamber	18 x 12.5 x 18 in.	18 x 19 x 18 in.	18 x 25.5 x 18 in.	37 x 25 x 21 in.	30 x 72 x 24.25 in.
	(46 x 32 x 46 cm)	(46 x 48 x 46 cm)	(46 x 65 x 46 cm)	(94 x 64 x 53 cm)	(72.2 x 182.9 x 61.6 cm)
Exterior	25 x 26 x 23.5 in.	25 x 33 x 23.5 in.	25 x 39 x 23.5 in.	39.8 x 34.5 x 25 in.	36 x 88.5 x 31.5 in.
	(64 x 66 x 60 cm)	(64 x 84 x 60 cm)	(64 x 99 x 60 cm)	(101 x 88 x 64 cm)	(91.4 x 224.5 x 74.3 cm)
<b>Electrical Specs</b>					
120 V (60Hz)	330 watts/8.3 amps	330 watts/8.3 amps	410 watts /8.9 amps	520 watts/4.3 amps	450 watts/12.1 amps
240 V (50/60Hz)	330 watts/6.6 amps	330 watts/6.6 amps	410 watts/7.0 amps	520 watts/2.2 amps	—
<b>Shelves</b>	1 supplied, 3 max	1 supplied, 6 max	2 supplied, 10 max	6 supplied, 36 max*	6 supplied, 30 max
<b>Catalog Number</b>					
120 V	<b>PR205045M</b>	<b>PR205055M</b>	<b>PR205065M</b>	<b>PR205075M</b>	<b>3971, solid door</b> <b>3973, glass door</b>
240 V	<b>PR205040M</b>	<b>PR205050M</b>	<b>PR205060M</b>	<b>PR205070M</b>	
<b>Shipping Weight</b>	150 lb/68 kg	165 lb/74.5 kg	190 lb/86.2 kg	215 lb/98 kg	685 lb/310.7 kg

\*18 shelves per door opening

Additional shelf kits for table top units: Catalog Number 13247S

Additional shelf kits for double door units: Catalog Number AY2076X1

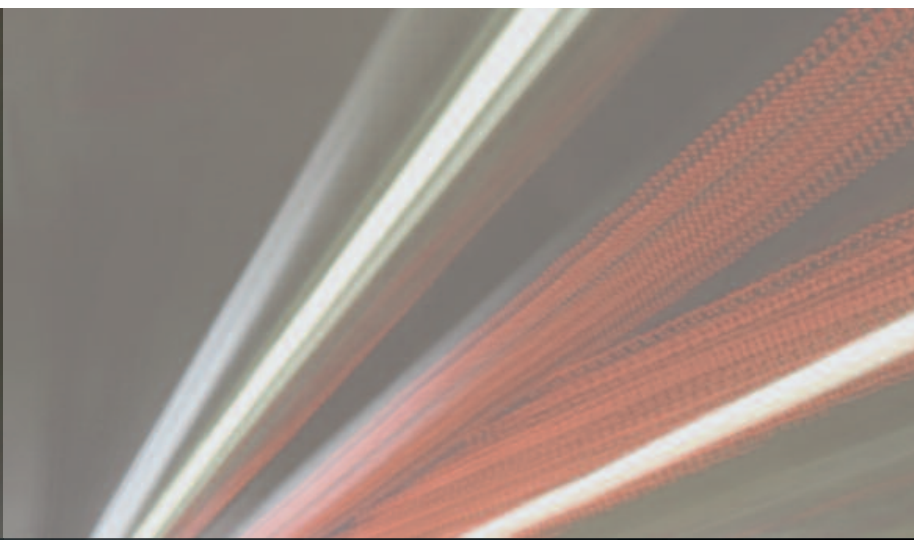
Additional shelf kits for large capacity units: Catalog Number 3166190

All 120V table top units are UL/cUL listed.

12 month warranty (parts and labor)







#### ***Gravity Convection Models***

- Three stackable, table-top units
- Outer door opens to 180° for unhindered access
- Choice of 120 V and 240 V versions
- Electrical outlet in all models



# Cytoline™ 1 macroporous microcarriers

Cytoline™ microcarriers are designed for use in packed, fluidized bed reactors for the culture of CHO and suspension cells intended for the production of recombinant proteins for therapeutic use. Cytoline microcarriers are macroporous and consist of a matrix based on polyethylene weighted with silica. The microcarriers yield high cell concentrations that can be sustained over long periods of time.

- Macroporous microcarriers
- High density cell culture
- Designed for fluidized bed cultures

## Macroporous microcarriers

Cells gain easy access to the interior of Cytoline due to the macroporous structure of the microcarriers (Figure 1). Inside Cytoline, cells are protected from both physical and environmental stresses, and are able to create their own micro-environment. This micro-environment increases metabolic communication between the cells and permits protein-free perfusion, which in turn decreases media costs, particularly in long-term continuous processes.

The polyethylene base matrix causes the microcarriers to be hydrophobic while the silica content gives Cytoline a slight negative charge. Cytoline is further primed by an NaOH wash during preparation to optimize performance, the effects of which are particularly noticeable in protein-free media.

## High density culture

Cytoline microcarriers offer both an external surface and an interior space that can be populated by anchorage-dependent cells or cells grown in suspension. Cell retention during perfusion is improved due to metabolic communication. As a result, cell density per reactor volume is high in contrast to when conventional microcarriers are used (Figure 2).



Fig 1. Scanning electron micrograph of Cytoline 1.

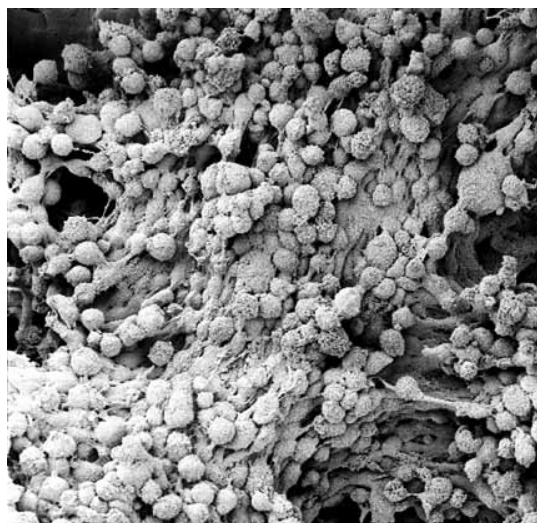


Fig 2. Close-up reveals the tight clustering of CHO cells on Cytoline 1.



## Designed for fluidized bed culture

**Cytoline 1** is optimized for the culture of CHO cells in fluidized beds. CHO cells attach well to Cytoline 1 and final cell densities are very high. These high densities are possible because of the high sedimentation rate of Cytoline 1, 120 to 220 cm/min. A high sedimentation rate enables the use of a high circulation rate, which ensures an adequate supply of oxygen to the reactor in order to maintain the high cell concentration.

## Good adhesion during long periods of culture

When culturing recombinant CHO cells using Cytodex™ microcarriers, the cells occasionally have a tendency to peel off the microcarriers after about 10 days in culture. When using Cytoline to culture CHO cells, there is no sign of cell detachment even after more than 60 days in culture.

## Characteristics of Cytoline microcarriers

- Steam sterilizable (*in situ*) 121 °C, 1 bar
- Alkali and acid resistant
- Lot-to-lot consistency
- No material of biological origin is used
- Suitable for immobilization and growth of adherent and non-adherent cell types
- Suitable for bioreactor systems that are in use in industrial environments, such as stirred tanks, airlift culture systems and fluidized beds
- Increases culture surface area when used in routine laboratory culture techniques, such as roller, spinner culture, or shaker flasks
- Lens-shape is advantageous for nutrient /oxygen supply diffusion
- Storage in the dark gives optimal shelf-life

## Specifications of Cytoline

Cytoline 1	
Sedimentation velocity (cm/min)	120–220
Length (mm)	1.7–2.5
Thickness (mm)	0.4–1.1
Density (g/cm <sup>3</sup> )	1.32
Pore size (µm)	10–400
Surface area (m <sup>2</sup> /g)	>0.3

## Ordering information

Product	Quantity	Code Number
Cytoline 1	50 mL	17-1268-01
Cytoline 1	500 mL	17-1268-02
Cytoline 1	5 L	17-1268-03

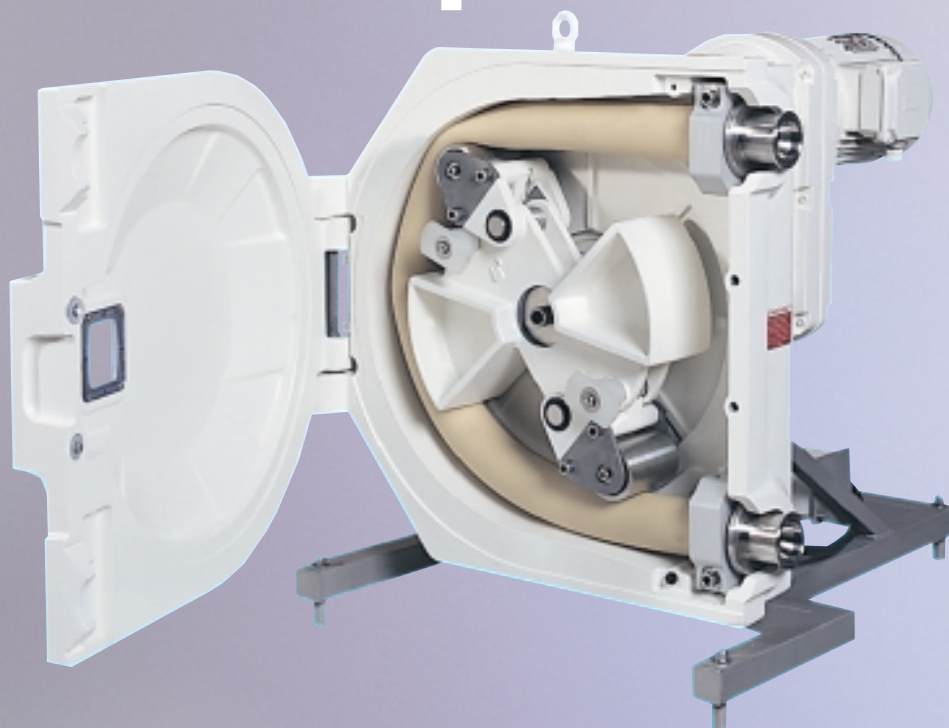


**WATSON**  
**MARLOW**  
*PUMPS*

S E R I E S

800

# High-flow **Hygienic** Pumps







## Put Watson-Marlow to work on your pumping problems

■ Watson-Marlow peristaltic pumps are problem solving pumps, best applied where other pump types fail or frustrate. Watson-Marlow works only through trained engineers, and, wherever you are, they will work with you, using the experience of meeting and solving literally thousands of pumping problems.

■ As the largest peristaltic pump manufacturer, with a range of pumps and tubing for flow rates from microlitres per hour to thousands of litres per hour, we stand every chance of helping you, but if we cannot we will tell you.

■ If we can help, we will do whatever is necessary to prove to you that it will do the job. Any product we recommend, we will stand by completely, and if it fails to meet the agreed need, then that will be our problem and not yours.

*Watson-Marlow leads the world in solving fluid handling problems with peristaltic pumping technology. The company is accredited for design and manufacture to ISO9001, and provides its customers with nothing less than the best products, service and knowledge. Watson-Marlow conducts its business ethically, and stands by its word, its recommendations and its products.*



## The benefits of peristaltic pumping

Compared to lobe pumps, diaphragm pumps, gear pumps and piston pumps, and every other type of pump, these are the advantages of Watson-Marlow pumps:


- 1 No contamination of the fluid.
- 2 No contamination of the pump.
- 3 Ideal for shear-sensitive and aggressive fluids.
- 4 Self-priming, dry-running.
- 5 No valves, seals or glands.
- 6 Automatic check valve action prevents backflow.




## PERISTALTIC PUMPS ARE IDEAL FOR


- Abrasive and aggressive fluids
- Beverage dispensing
- Cell culture
- Fermentation
- Filtration and separation
- Food processing
- Industrial chemicals
- Inks and pigments
- Pharmaceuticals
- Photographic solutions
- Water treatment



### Purchasers in the EU please note

825 and 840 pumpheads are specially designed for connection by the user to gearboxes of the users choice and they do not carry the  mark. These pumpheads are supplied with "Declarations of Incorporation" as required by the EU Machinery Directive.


The B/RA and the RA bare shaft pumps carry the  mark, but the complete motorised pump assembly must also carry the assembler's  mark in addition to the Watson-Marlow  mark.

Customers in EU member States must note that self assembly of machines carries with it the responsibility to ensure that the final working assembly complies with all the relevant EU safety directives and the assembler's  mark is affixed on completion.

### Common conversions

To go from	Multiply by	To get to
bar	14.50377	psi
inches	25.4	mm
deg C	(1.8 x C) + 32	deg F
gal (IMP)	4.5460	litres
gal (US)	3.7854	litres
gal (IMP)	1.20095	gal (US)
gpm (US)	0.22712	m <sup>3</sup> /hr
gpm (IMP)	0.27276	m <sup>3</sup> /hr
inches Hg	1.1329	ft H <sub>2</sub> O
inches Hg	0.4912	psi
kg/cm <sup>2</sup>	14.22334	psi
kW	1.341	hp
kPa	0.14504	psi
N/m <sup>2</sup>	1.0	Pa
Pa	0.00001	bar
litre	0.0001	m <sup>3</sup>
kgcm	0.098	Nm

### Standards

 Conforms to all relevant Directives

**IEC 335-1** is the International Electrotechnical Commission standard dealing with the "Safety of household and similar appliances, general requirements". Equivalents are BS3456: Part 101 and DIN VDE 0700: Part 1).

**EN60529** is the European Norm standard dealing with the "Classification of degrees of protection provided by enclosures for rotating machines. Equivalents are BS 4999: Part 105, IEN 60034: Part 5, and DIN VDE 0530: Part 5. IP numbers (such as IP34, IP42, IP55) indicate the degree of ingress protection of the product, with the first digit indicating protection against the ingress of objects, and the second digit indicating the degree of protection against the ingress of water.

**EN60204-1** is the European Norm covering "The Safety of Machines - Electrical Equipment of Machines".

**EN61010-1** is the European Norm covering "Safety requirements for electrical equipment for measurement, control and laboratory use".

**EN50081-1** is the European Norm covering "Electromagnetic compatibility - Generic Emission standard - Residential, commercial and light industry".

**EN50082 - 1** is the European Norm covering "Electromagnetic compatibility - Generic Immunity standard - Residential, commercial and light industry".









### Spare parts availability

Watson-Marlow's policy is to provide spare parts for all products for a minimum of eight years from discontinuation. For major products, this period is extended to twelve years. The ability to implement this policy is not entirely within Watson-Marlow's control and cannot be guaranteed, but every effort will be made to honour this policy.

# 825

## 25mm High-flow, hygienic pump

# 825

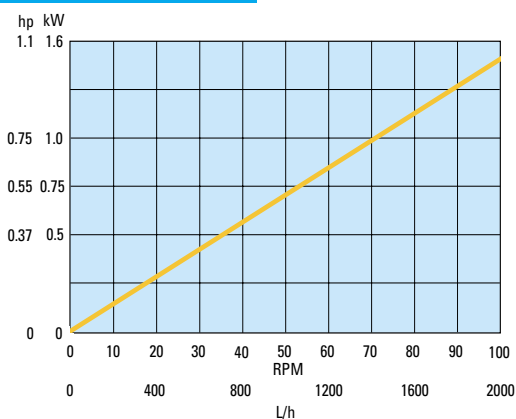
-  Provides 2000 litre/hr (0.33 litre/rev)
-  Twin stainless steel rollers
-  Hinged door with only two captive bolts makes changing tube extremely simple and safe
-  Use USP Class VI Bioprene tubing for 2 bar (29 psi) and 3.5 bar (51 psi) operation
-  Tube elements complete with hygienic connectors
-  Tubing in extended element lengths for fitting customers own connectors
-  Bareshaft pumpheads to take a range of foot-mounted motors with flexible couplings
-  Pumpheads to accept IEC B5 output flange-mounted gear motors



### Standards

BS800, IEC 335-1,  
EN60529 (IP55) CE

### 825R or B/R Motor power/tube life



### Ordering information

#### Pumps

825B/R	Baseplate mounted bareshaft pumphead	<a href="#">080.2500.000</a>
825RA	Pumphead with bareshaft adaptor (no baseplate)	<a href="#">083.2510.000</a>

#### Bioprene tubing for 825 pumps

Tube bore				
25mm	1"	2 bar TM tube element	770mm length	<a href="#">088.0250.F0M</a>
25mm	1"	3.5 bar TH tube element	770mm length	<a href="#">088.0250.F0H</a>
25mm	1"	2 bar TM tube element	2.3m length	<a href="#">088.0250.00M</a>
25mm	1"	3.5 bar TH tube element	2.3m length	<a href="#">088.0250.00H</a>

#### Connectors for 825 pumps

825CT	Triclap hygienic connector	1"	<a href="#">089.0250.00T</a>
825CS	SMS hygienic connector		<a href="#">089.0250.00S</a>
825CI	IDF/ISS hygienic connector		<a href="#">089.0250.00R</a>
825CQ	Quick coupling (MIL-C-2748F)	1"	<a href="#">089.0250.00Q</a>
825CR	Quick RJT (BS1864) connector		<a href="#">089.0250.00R</a>

#### Tube lubricant

400g	Translucent food machinery grease (USDA-H1) approved	<a href="#">098.0005.000</a>
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## Drive options

A number of motor/gearbox options and control options are available for these pumps including fixed and variable speed with mechanical variations. The options include inverter control enabling 4-20mA and 0-10V process control, remote stop/start and reverse, and frequency output.

## Configurations

All 800 series pumps are available from Watson-Marlow as complete, ready to run, motorised pumps with a two year warranty.

Each pump supplied by Watson-Marlow is a fully assembled, ready-to-run unit, or a B/RA or an RA bare shaft, ready-to-drive pumphead, which complies with all relevant EU (European Union) safety directives and carries the relevant  mark or declaration of incorporation.

## Fixed speed pumps

825 and 840 pumps can be supplied as a complete speed units fitted with an IP55 motor and gearbox in 4 different speeds.

825FB/R pump speeds are 22, 38, 68 and 98 rpm. 840FB/R pump speeds are 22, 39, 62 and 100 rpm.

## Variable speed inverter controlled pumps

We supply complete electronically variable speed pumps with two maximum speeds with a 5:1 reduction ratio. The minimum flow rate will be approximately 20% of the maximum achievable.

Watson-Marlow have selected as standard accessories, single phase input/three phase output inverters for use with the 800 series pumps (maximum power requirement 1.5KW, for use with the 825 pumps and 2.2KW for use with 840 pumps).

All Watson-Marlow pumps are available fitted with motorised geared units to suit the users own choice of inverter.

## Mechanically variable speed pumps

All Zone 1 Exd 800 series pumps and those rated above 2.2KW are fitted with belt driven mechanically variable speed gearboxes and IP55 standard motors.

## Explosion-proof pumps

All pumps are available fitted with Exd IIB T4 specification explosion-proof motors that meet all EU explosion-proof standards. Specially selected combinations of explosion-proof motors and electronic inverters are available. These inverters are not fitted with explosion-proof casework and therefore must be sited outside the hazardous area.

## Accessories

Pulsation dampers will be required for certain applications. Watson-Marlow will supply pulsation dampers from the Flowguard range.

Tube failure detectors are available to order.

A choice of feet or castors for fitting to 825 and 840 pump frames are available.



# 800

## Connectors

800 series pumps may be used with either tube elements fitted with hygienic connectors or extended element lengths. A choice of connector types is offered, including Triclamp (Triclover and 3A compatible), IDF and SMS sanitary, and RJT. These are constructed from 316 stainless steel and are totally autoclavable.

## Operating and storage temperatures

Unless otherwise stated, all pumps listed in this brochure may be operated at ambient temperatures between 5C and 40C (41F and 104F). They may be stored at temperatures between -40C and 70C (-40F and 158F), but allow time for acclimatisation before operating.



## Tubing

The Watson-Marlow 800 series pumps use a Bioprene tube which meets USP and NFS Class VI requirements. Bioprene also complies with FDA 21 CFR 177.260 and meets USDA standards for food handling.

### High pressure applications

For specific higher pressure applications, use 3.5 bar "TH" tubing elements for intermittent periods only. Do not use quick connectors for this process. Tubing previously used as a pumping element should not be used as a transfer section.

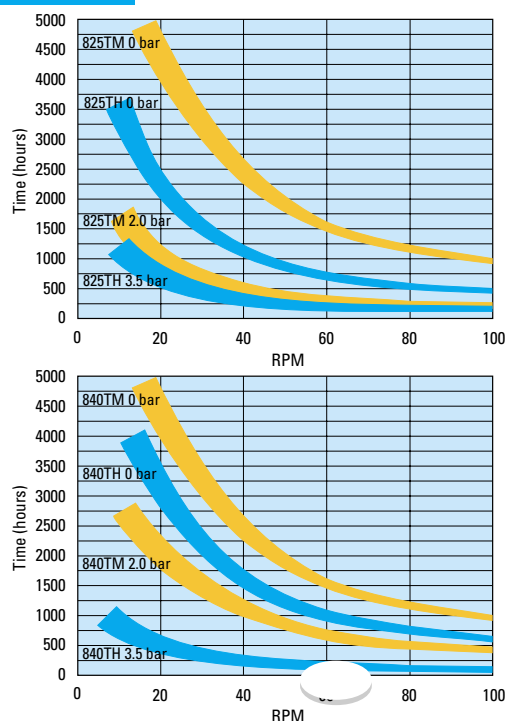
### Tube lubricant

Translucent food machinery grease (USDA-H1 approved for food contact) is available for 800 series tubing to prolong tube life.

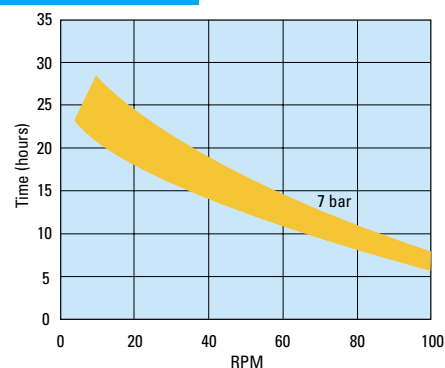
## Flow rates

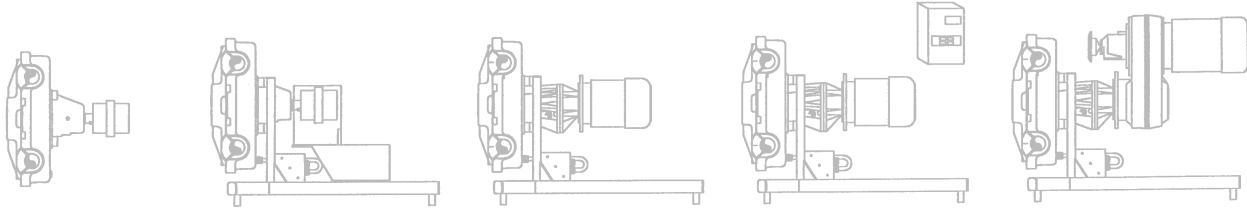
All flow rates quoted in this brochure were obtained pumping water at 20C (68F) with zero suction and delivery heads.

## Tube life



## High pressure tube life





825RA

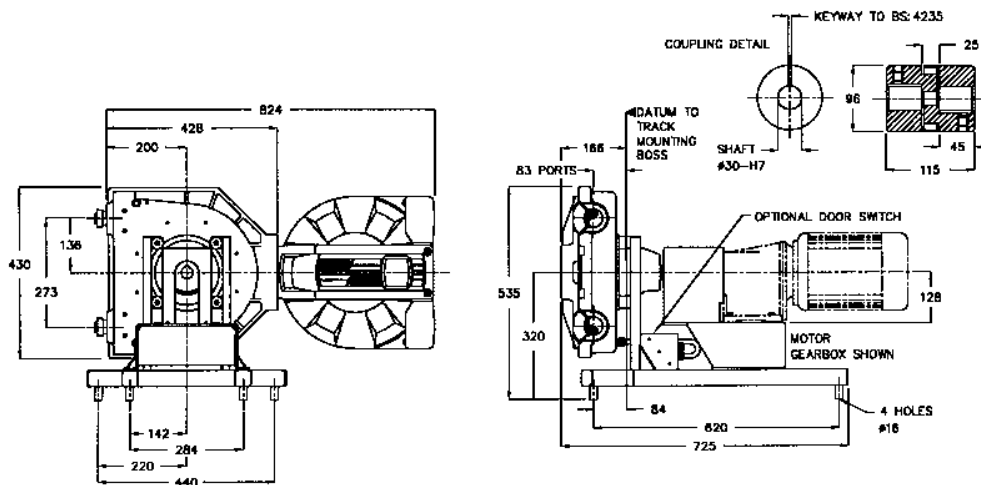
825B/RA

825FB/R or DFB/R

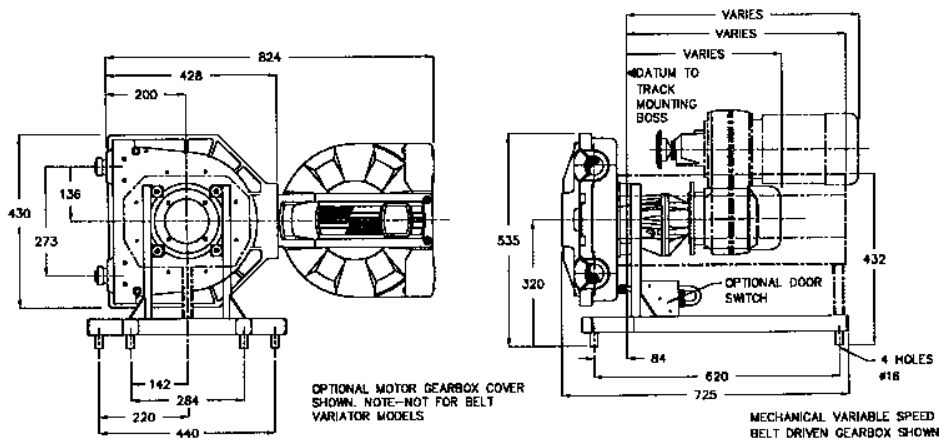
825VBI/R

825VB/R or DVB/R

## Dimensions mm



825B/RA



825FB/R, 825VBI/R, 825DFB/R, 825DVB/R

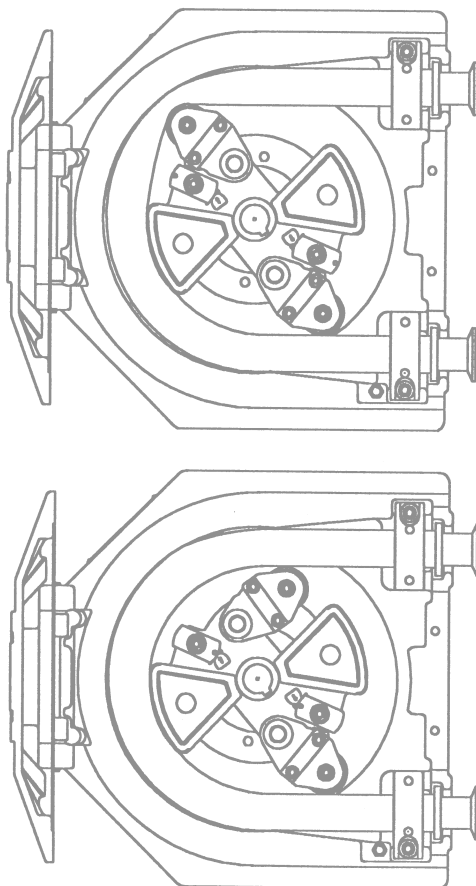
# 800

## Materials of construction

Description	Material
Pumphead body	Aluminium alloy with epoxy polyester powder coat finish
Pumphead door	Aluminium alloy with epoxy polyester powder coat finish
Pumphead rotor	Aluminium alloy
Rotor rollers	316 stainless steel
Frame	Stainless steel 304L
Optional motor housing	Stainless-steel 304L
Door fixings	Stainless steel
Motor fixings	Zinc plated high tensile steel bolts, stainless steel nuts and washers
Frame fixings	Stainless steel

### Paint specification

The 800 series epoxy polyester powder coating is based on a mixed polymer thermosetting resin designed to combine the durability associated with epoxies with the enhanced UV and heat stability associated with polyesters. This results in a high quality finish which is abrasive and impact resistant.



### Installation drawings

Installation drawings of all 800 series pumps are available on request in A3 size. Reproducible drawings are also available for a modest fee.

### Custom-built pumps

Watson-Marlow Limited's team of technical support engineers is available to advise and quote for your custom-built pumps with electric, hydraulic or pneumatic motors, trolley mounting, stainless steel baseplates etc.

### CIP

The 800 series pumphead incorporates retractable roller mechanisms which allow clean-in-place or steam-in-place hose cleaning. The roller mechanisms are simply unlocked and disengaged from the standard pumping position, providing maximum cleaning capacity via a free flow of cleaning agents through the hose.

### SIP

The 800 series can be steamed-in-place at 135°C (2 bar) for a duration of one hour. Allow 15 minutes for pump to return to ambient temperature prior to restarting pump.





## Flow rates

Up to 8,000 litres/hour depending upon motor speed.

## Pressures

Up to 3.5 bar continuous operation governed by motor sizing.

## Drive options

Fixed speed, variable speed (inverter or mechanical variator), all drives available with Exd options. Contact Watson-Marlow Technical Support for further information.

## Duty rating

24 hour governed by motor sizing.

25mm hygienic pumps



40mm hygienic pumps



*The 800 series pumps have been designed for hygienic pumping up to 8000 litre/hour and pressures to 3.5 bar (51 psi), in the biotechnology, pharmaceutical and food industries. Pumpheads are constructed of aluminium with an epoxy powder coated white finish and they offer true CIP (clean-in-place) and SIP (steam-in-place) as the rollers retract to a non-occluding position, allowing a complete flow-through for cleaning.*

*All pumps are mounted on a stainless steel frame with the option of a brushed stainless steel motor housing, where belt variators are not required.*

*Watson-Marlow's focus has been to minimise the total cost of ownership over the lifetime of the pumps by making them reliable, long-lasting, and simple to use and maintain. Downtime is the true enemy of productivity, and so minimising downtime and whatever essential maintenance is required rewards our care and your choice of pump.*

*For instance and (uniquely), access to the tube is through a hinged door rather than a lift-off cover, and the door is secured by two captive bolts rather than sixteen or twenty loose ones, making tube changing fast, simple and safe.*

*Bioprene tube elements with hygienic connectors are autoclavable and can be fitted quickly and cleanly.*

*Where hygiene and system up-time is valued, there is no better pump than a Watson-Marlow 800 series.*

**WATSON  
MARLOW**

## Unmatched warranties

The high quality of the design and manufacture of Watson-Marlow products allows us to offer international warranties far exceeding those available for other pump types.

All 800 series pumps carry a two year warranty.





# WATSON MARLOW PUMPS

The information contained in this document is believed to be correct but Watson-Marlow Limited accepts no liability for any errors it contains, and reserves the right to alter specifications without notice.

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## S E R I E S

### 100/200

#### Low-flow pumps

100 series flow rates from 0.01 to 53 ml/min. Single channel. Fixed and variable flow rates.



#### Multi-channel pumps

200 series flow rates from microlitres to 32 ml/min, through up to 32 channels. Manual and auto-control.

● HB 0065



### 300

#### Low-cost pumps

Flow rates from 0.2 to 2,000 ml/min. Fixed and variable speeds.

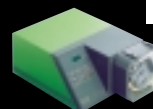
● HB 0060



### 500

#### Microprocessor controlled pumps

Flow rates from 0.01 to 2,200 ml/min. 1 to 48 channels. Manual, auto and digital control.



#### IP55 rated pumps

Washdown pumps for tough environments, and pneumatic or baseplate-mounted pumps with Exd motors for hazardous areas. Flow rates to 2.2 litre/min.

● HB 0062

### 600

#### Mid flow pumps

Cased pumps or baseplate-mounted pumps for flows up to 15.8 litre/min (4.2 US gpm). Fixed or variable speed and single channel.

● HB 0063



### 700

#### High-flow pumps

IP55 rated pumps with manual or auto control and baseplate-mounted industrial pumps provide flows to 2,000 litre/hr (8.8 US gpm). Single or twin channel.

● HB 0060

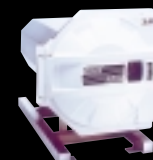


### 800

#### High-flow hygienic pumps

Use Bioprene USP Class VI tubing for flow rates up to 8,000 litre/hr (35 US gpm), and pressures up to 3.5 bar (50 psi). True CIP and SIP facilities ideal for biotech, pharmaceutical and food industries. Single channel.

● HB 0057



### 900

#### High-flow, high-pressure pumps

Use reinforced tubing in five different materials for flow rates up to 10,000 litre/hr and pressures up to 15 bar (210 psi). Single channel.

● HB 0056



## Dispensers & Robotic fillers

For repetitive and accurate dosing and dispensing duties. Straightforward stop/start volumes up to one litre in five seconds or precision, balance-controlled dispensing.

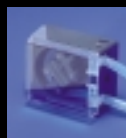
● HB 0039



## OEM pumps

Pumpheads for use with customers' own drives or plate-mounted pumpheads and motors. Compact, easy to install and to use. Single and multi-channel with flow rates up to 2,700 ml/min.

● HB 0076



## Tubing

Twelve different materials and over 50 sizes of tubing for the complete range of Watson-Marlow pumps. Full chemical compatibility tables with 450 fluids tabulated. Performance and physical data.

● HB 0065



Members of the Spirax-Sarco Engineering Group

Watson-Marlow is a registered trademark of Watson-Marlow Limited.

Publication ref: HB 0057

● These brochures describe the full Watson-Marlow range of peristaltic pumps, tubing and accessories.

ST DESIGN

## BIOPRENE TUBING

Thermoplastic elastomer tubing

Highly resistant to oxidising agents

### Validated processes

Bioprene peristaltic pump tubing is fully approved for food and pharmaceutical applications. Validated processes are supported. Like Marprene, Bioprene lasts at least 10 times as long as tubing in other materials. Long-life tubing means stoppages for a tube change are rare events, easily fitted in with general maintenance.

### Easy sterilisation

Clean-in-place and steam-in-place (with tubing elements) are no problem for the tube that has everything.

USP, ISO and FDA approvals matter in maintaining validated processes. Bioprene peristaltic pump tubing offers long life and chemical resistance, and carries the widest range of safety approvals

- USP Class VI FDA approvals
- Fully documented bio-compatibility and comprehensive validation pack
- Safe for use in biomedical applications
- Ex-stock availability
- Comprehensive stock of a wide range of sizes
- UV opaque
- Fully weldable
- No odour
- Low extractables
- Suitable for repeated autoclave cycles

**Watson-Marlow Bioprene: the best peristaltic pump tube life with excellent chemical resistance**



## For long life and chemical compatibility

Bioprene offers exceptionally long life and resistance to a wide range of chemicals. It is a first choice for applications including metering tablet-coating materials, pH control and media feed in fermentation and bioreactor metering, as well as pharmaceutical handling, dispensing, metering, transfer and filtration.

**General industrial long-life transfer tube. Highly resistant to oxidising agents such as ozone, peroxides and sodium hypochlorite.**

Bioprene	Typical values
Material	Thermoplastic elastomer
Colour / transparency	Beige / opaque
Spallation	Fair
Life, hours	313/314, 520R pumpheads: 10,000; 620R pumphead: 6,000
Certification	FDA regulations 21 CFR 177.2600 for contact with aqueous food
Sterilisation methods	Gamma, autoclave, EtO. CIP, SIP: refer to Watson-Marlow for details
Operating temperature	5C-80C
Hardness, shore A (5 sec)	64 shore: 66; 73 shore: 74; 87 shore: 88
Specific gravity	64 shore: 0.97; 73 shore: 0.96; 87 shore: 0.95
Tear B, ppi	-
Ultimate tensile strength, psi	64 shore: 1007; 73 shore: 1410; 87 shore: 2263
Elongation at break, %	64 shore: 473; 73 shore: 510; 87 shore: 547
Tensile stress at 100% elongation, psi	64 shore: 367; 73 shore: 525; 87 shore: 987
Compression set, %	-
Weather resistance	Excellent
Sunlight resistance	Excellent
Gas permeability, O <sub>2</sub> cc.cm x 10 <sup>-4</sup> / cm <sup>2</sup> .sec.atm	5.8
Gas permeability rating	Fair
Water absorption	Good
Odour	Excellent
ASTM methods	Hardness: ASTM D 2240; Specific gravity: ASTM D 792; Tear B, Ultimate tensile strength, Elongation at break, Tensile stress at 100% elongation: ASTM D 412

Bore		Wall		Coil size	Part number	Coil size	Part number	Coil size	Part number
inch	mm	inch	mm						
1/16	0.5	1/16	1.6	50ft / 15m	903.0005.016	16ft / 5m	903.0005.J16	500ft / 152m	903.0005.R16
1/32	0.8	1/16	1.6		903.0008.016		903.0008.J16		903.0008.R16
1/16	1.6	1/16	1.6		903.0016.016		903.0016.J16		903.0016.R16
3/32	2.4	1/16	1.6		903.0024.016		903.0024.J16		903.0024.R16
1/8	3.2	1/16	1.6		903.0032.016		903.0032.J16		903.0032.R16
3/16	4.8	1/16	1.6		903.0048.016		903.0048.J16		903.0048.R16
1/4	6.4	1/16	1.6		903.0064.016		903.0064.J16		903.0064.R16
5/16	8.0	1/16	1.6		903.0080.016		903.0080.J16		903.0080.R16
					903.0005.024		903.0005.J24		
					903.0008.024		903.0008.J24		
1/16	1.6	3/32	2.4	50ft / 15m	903.0016.024	16ft / 5m	903.0016.J24	400ft / 122m	903.0016.R24
1/8	3.2	3/32	2.4		903.0032.024		903.0032.J24		903.0032.R24
3/16	4.8	3/32	2.4		903.0048.024		903.0048.J24		903.0048.R24
1/4	6.4	3/32	2.4		903.0064.024		903.0064.J24		300ft / 91m903.0064.R24
5/16	8.0	3/32	2.4		903.0080.024		903.0080.J24		200ft / 61m903.0080.R24
3/8	9.6	3/32	2.4		903.0096.024		903.0096.J24		150ft / 46m903.0096.R24
					903.0048.032		903.0048.I32		
1/4	6.4	1/2	3.2		903.0064.032		903.0064.I32		200ft / 61m903.0064.R32
3/8	9.6	1/2	3.2		903.0096.032		903.0096.I32		150ft / 46m903.0096.R32
1/2	12.7	1/2	3.2		903.0127.032		903.0127.I32		
3/8	15.9	1/2	3.2	903.0159.032	903.0159.I32	Custom sizes are available to order, as are other specific requirements			
-	8.0	-	4.0	903.0080.040	903.0080.I40				
-	12.0	-	4.0	903.0120.040	903.0120.I40				
-	16.0	-	4.0	903.0160.040	903.0160.I40				
3/8	9.6	3/16	4.8	903.0096.048	903.0096.I48				
1/2	12.7	3/16	4.8	903.0127.048	903.0127.I48				
3/8	15.9	3/16	4.8	903.0159.048	903.0159.I48				
3/4	19.0	3/16	4.8	903.0190.048	903.0190.I48				
1	25.4	3/16	4.8	903.0254.048	903.0254.I48				

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**WARNING** These products are not designed for use in, and should not be used for, patient-connected applications.

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Value for life





## Sartopore® 2 0.2 µm

Sterilizing Grade Filter Cartridges



### Description

Sartopore® 2 0.2 µm rated sterilizing grade filter cartridges are designed for filtration of a broad range of pharmaceutical products where compliance with cGMP requirements has to be fulfilled. Sartopore® 2 cartridges feature a unique hydrophilic heterogeneous double layer Polyethersulfone membrane with broad chemical compatibility, high thermal resistance and higher throughput and flow-rate than any other sterilizing grade filter cartridge.

### Applications

Typical applications include sterilizing grade filtration of:

- Therapeutics
- Biological Fluids
- Ophthalmics
- SVPs, LVPs
- Antibiotics
- WFI
- Chemicals
- Cleaning and sanitizing agents
- Bulk pharmaceutical products

### Compatibility

The polyethersulfone membrane is compatible with a pH range from pH 1 to pH 14 and unaffected by steam sterilization cycles making Sartopore® 2 cartridges ideal for filtration of solutions with high | low pH and for SIP | CIP-cycles.

### Performance

Sartopore® 2 cartridges provide an exceptionally high total throughput by fractionated filtration due to the "built-in prefiltration" of the 0.45 µm membrane. The asymmetric pore structure of the polyethersulfone membrane provides high flow rates at low pressure drops.

### Wettability

Sartopore® 2 cartridges can be easily wetted out for integrity testing even after drying at 80 °C for 12 hours.

### Microbiological Retention

Sartopore® 2 filter cartridges are fully validated as sterilizing grade filter elements according to HIMA and ASTM F-838-83 guidelines.

### Quality Control

Each individual element is integrity tested by diffusion and bubble point test prior to release, assuring absolute reliability.

### Documentation

Sartopore® 2 cartridges are designed, developed and manufactured in accordance with an ISO 9001 certified Quality Management System. A Validation Guide and Extractables Guide are available for compliance with regulatory requirements.

### Specifications

#### Materials of Construction

Prefilter Membrane:	Polyethersulfone, asymmetric
Endfilter Membrane:	Polyethersulfone, asymmetric
Support Fleece:	Polypropylene
Core:	Polypropylene
End Caps:	Polypropylene
O-Rings:	Silicone (optional EPDM or Viton)

#### Pore Size

0.45 µm + 0.2 µm

#### Available Sizes | Filtration Area

Size 1	10"	0.6 m <sup>2</sup>   6 ft <sup>2</sup>
Size 2	20"	1.2 m <sup>2</sup>   12 ft <sup>2</sup>
Size 3	30"	1.8 m <sup>2</sup>   18 ft <sup>2</sup>

#### Available Adapters | Connectors

21, 25, 27, 28

#### Operating Parameters

Max. Allowable	5 bar   75 psi at 20 °C
Differential Pressure:	2 bar   29 psi at 80 °C
Max. Allowable Back Pressure:	2 bar   29 psi at 20 °C

## Specifications

### Extractables

Sartopore® 2 0.2 µm rated filter cartridges meet, or exceed the requirements for WFI quality standards set by the current USP.

### Regulatory Compliance

100% Individually integrity tested

Integrity test correlated to HIMA/ASTM F 838-83 Bacteria Challenge Test

Non-pyrogenic according to USP Bacterial Endotoxins

Passes USP Plastics Class VI Test

Non-fiber releasing according to 21 CFR

## Sterilization

### In-Line Steam Sterilization:

134 °C, 20 min. at max differential pressure of 0.5 bar | 7.25 psi

### Autoclaving:

134 °C, 2 bar | 29 psi, 30 min

### Sterilization Cycles

In-Line Sterilization: Min. 25

Autoclaving: Min. 25

## Technical References

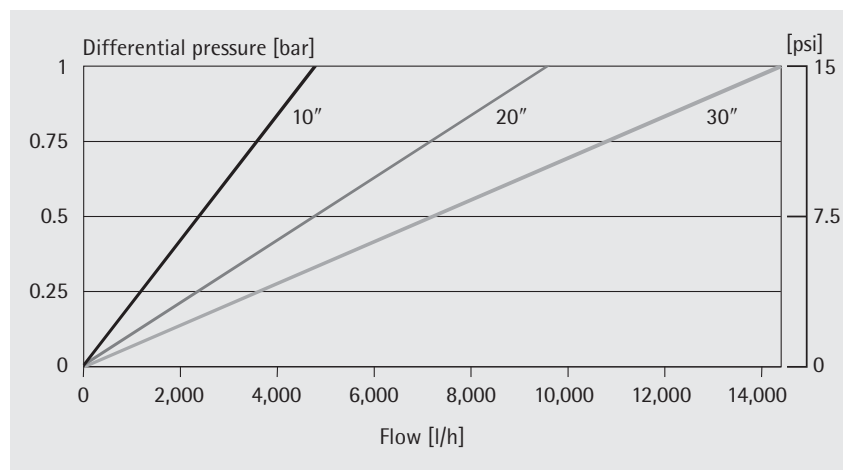
### Validation Guide:

SPK 5732-e

### Extractables Guide:

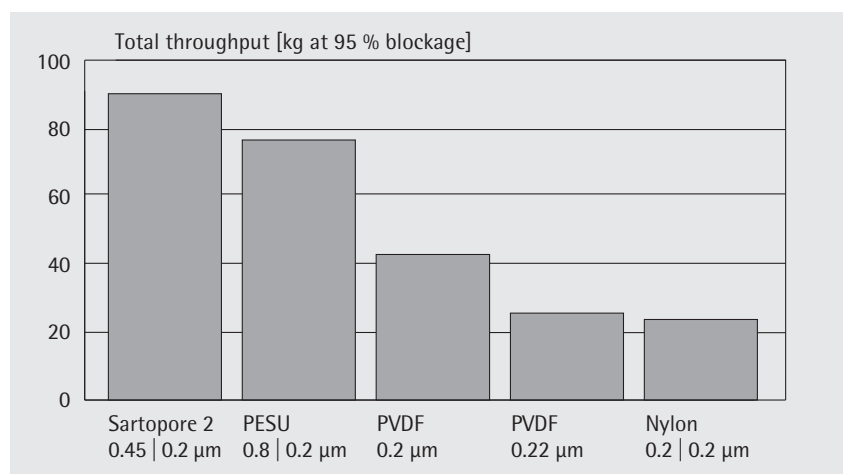
SPK 5731-e

Water Flow Rates for 10", 20" and 30" Cartridges



Standardized at 20 °C

Total Throughput Comparison



10" Cartridge format

## Ordering Information

Order Code.	Pore size [µm]	Test Pressure [bar   psi]	Max. Diffusion [ml/min]	Min. B.P. [bar   psi]
544**07H1	0.2	2.5   36	18	3.2   46
544**07H2	0.2	2.5   36	36	3.2   46
544**07H3	0.2	2.5   36	54	3.2   46

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Publication No.: SPK2030-e08066  
Order No.: 85030-511-67

# WAVE Bioreactor™ 2/10 system

Expand your horizons in cell therapy culture



- Functionally closed system
- Easy scale up and scale out
- No need for incubator
- Cost-effective





## 1 The right system for your suspension cells

The WAVE Bioreactor 2/10 system is a widely cited cell culture platform based on a single-use presterilized bag called a Cellbag™ bioreactor. The rocking motion of the platform induces waves to mix and transfer oxygen to the culture medium to create an optimal environment for cell growth. Wave-induced mixing in a cGMP-compliant environment enables time-efficient, cost-effective, and scalable expansion of cells suitable for cell therapy research applications.

- Achieve high-density expansion of cells such as T, TIL, and NK cells (1,2,3)
- Maintain the phenotypic and functional characteristics you expect from static culture (2)
- Expand cells suitable for autologous transplantation in adoptive immunotherapy applications (1,3,4,5,6)
- Integrate into clinical trial studies using functionally closed Cellbag bioreactors free of animal products (3)

**Table 1.** Cell densities achieved using the WAVE Bioreactor 2/10 system

Cell type	Yield (cells/mL)
T	$1.5 \times 10^7$ (5)
TIL	$1.5 \times 10^7$ (2)
NK	$9.8 \times 10^5$ (3)

## 2 Better by design

### Minimal cross-contamination in a functionally closed system

Scale-up from 250 mL to 5 L of culture in a single Cellbag bioreactor to achieve your target cell concentrations without the need for a laminar flow cabinet (3).

Sampling, monitoring, and additions all take place within in a functionally closed environment removing the risk of contamination associated with processes where multiple bags or flasks are required (4).

### Customizable to meet your changing needs

Cellbag bioreactors can be customized with a range of specialized fittings and ports to support your specific project needs.

For more information,  
visit [www.gelifesciences.com/waveinfo](http://www.gelifesciences.com/waveinfo)

### Higher cell densities in a low-shear environment

Set the rocking motion of the Cellbag holder to different angles and different rates to induce waves that provide efficient mixing and oxygen transfer in a low-shear environment. The resulting homogeneous culture enables higher cell densities to be achieved in a shorter period of time than is possible with static bags (2,3,5).

### 3 Take control of process parameters

Gain the flexibility needed to support your cell culture requirements with the modular format of the WAVE Bioreactor 2/10 system.

Set, maintain, and control CO<sub>2</sub> concentration using a CO<sub>2</sub>/Air Mix Controller and automate feeding of cultures and removal of waste products with a Perfusion Controller. Integral temperature control means there is no need for an incubator.



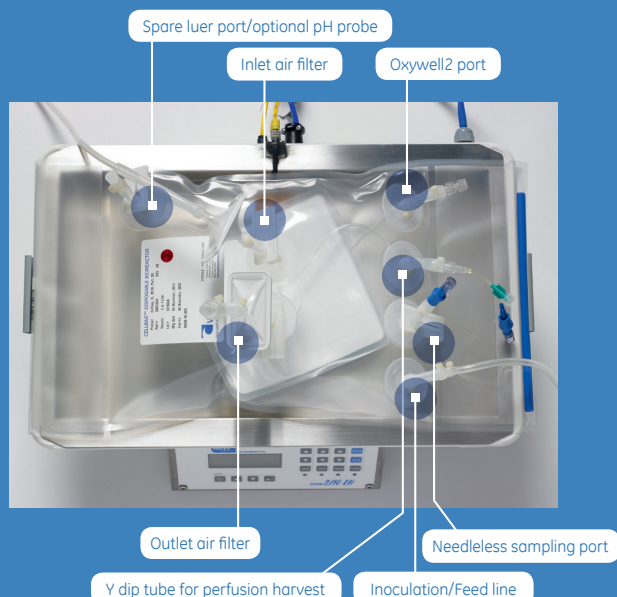
**CO<sub>2</sub>/Air Mix Controller:** An aeration system with CO<sub>2</sub> sensor provides and controls a continuous supply of CO<sub>2</sub>-conditioned air to the Cellbag bioreactor.



**Perfusion Controller:** Includes a scale and peristaltic feed and harvest pumps to minimize culture volumes through weight-based perfusion.

### 4 Easy scale-out for multiple cultures

Scale-out using the WAVE Bioreactor 2/10 system increases productivity and reduces labor costs compared to static cultures. Perform multiple applications simultaneously by linking up to 10 systems together and monitor using the optional PCDAQ software on a single PC.



**Cellbag bioreactor:** Typical Cellbag bioreactor configuration. Other designs are available. A range of ports enable sampling in a functionally closed environment. Optional ports and sensors allow measurement of critical parameters such as pH and DO. Maximum cell culture volume is half the total Cellbag bioreactor volume allowing air space for optimized agitation and gas transfer.



Gentle rocking of the Cellbag bioreactor ensures efficient mixing of culture media.

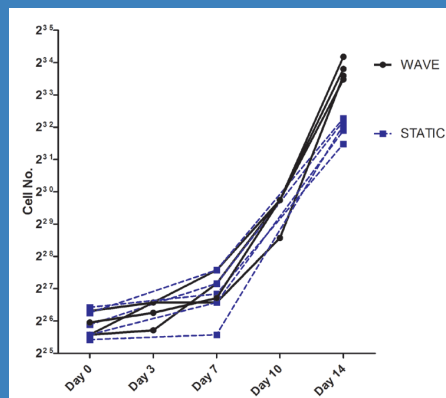


## 5 More cells for less with the Perfusion Controller

Automated media exchange and working volumes of up to 5 L in a single disposable Cellbag bioreactor make the WAVE Bioreactor 2/10 system easier to use and considerably less laborious than static cultures where multiple bags or flasks are usually needed (1,2,3,4,5,6).

### Increase expansion efficiency

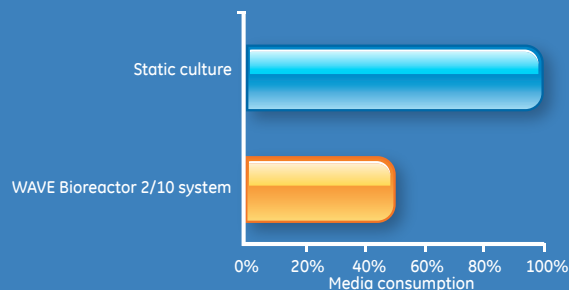
Continuous noninvasive mixing of preactivated cells results in higher yields of final product compared to static culture (1,2,3,4,5,6).



Growth profile of nine independent TIL cultures. Cells grown in the Cellbag bioreactor exhibit significantly higher proliferation rates and absolute cell numbers than cells grown in static conditions. (Reproduced with permission) (2).

### Minimize media consumption

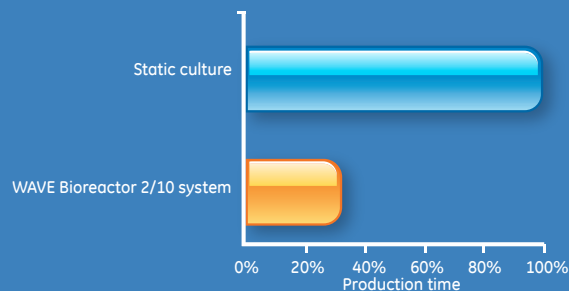
Optimize nutrient concentration and minimize the amount of culture media required using the Perfusion Controller (2,3). Automatic media exchange and scale-up using a single Cellbag bioreactor reduces media consumption by 50% compared to static culture.



Typical percentages of media consumption for an application with TIL cells. Data based on an article by Sadeghi, A., *et al* (4).

### Reduce hands-on-time and lower costs

Reduce production time by up to 60% compared to static cultures using the WAVE Bioreactor 2/10 system with perfusion (2,3,4,5) and lower costs using a single Cellbag bioreactor for scale-up when multiple bags or flasks are often needed. The amount of labor required to process and harvest the higher densities of viable cells achieved with automated media exchange is also reduced.



Typical percentages of production time required for an application with TIL cells. Data based on an article by Sadeghi, A., *et al* (2).

## Ordering information

Description	Code number
Cellbag 500mL (BC10, Basic)	28-9480-89
Cellbag 2L (BC10, Basic)	28-9378-00
Cellbag 2L (BC10, DO)	28-9377-99
Cellbag 2L (BC10, Perfusion, DO)	28-9376-52
Cellbag 10L (BC10, Basic)	28-9378-01
Cellbag 10L,(BC10, Perfusion, DO)	28-9376-62
Cellbag 20L (BC10, Basic)	28-9378-02
Cellbag 20L (BC10, DO)	28-9377-96
Cellbag 20L (BC10, Perfusion, DO)	28-9376-64
WAVE Bioreactor BASE2/10EH	28-9377-86
LID2/10W – OPAQUE	28-9376-33
Perfusion Controller PERFCONT2E	28-9884-64
WAVE Bioreactor BASE20/50EHT-L 100-120 V	28-9616-88
WAVE Bioreactor BASE20/50EHT-L 220-240 V	28-9616-89
WAVE Bioreactor BASE20/50EHTD-L 100-120 V	28-9436-72
WAVE Bioreactor BASE20/50EHTD-L 220-240 V	28-9436-95
WAVE Bioreactor BASE20/50EHT-CO2-L 100-120 V	28-9436-96
WAVE Bioreactor BASE20/50EHT-CO2-L 220-240 V	28-9436-97

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# WAVE Bioreactor™ 500/1000 system

WAVE Bioreactor 500/1000 system (Fig 1) is part of GE Healthcare Life Sciences' ReadyToProcess platform of ready-to-use products. The system is a cell culture device for the production of recombinant proteins in mammalian and insect cell lines in batch, fed-batch, and perfusion culture. Culture medium and cells are loaded into a single-use, presterilized bag known as the Cellbag™ bioreactor. The Cellbag bioreactor is placed on an electric rocking base. The rocking motion of this base induces waves in the cell culture fluid within the Cellbag bioreactor to provide efficient mixing and gas transfer. The resulting environment within the Cellbag bioreactor can easily support  $1 \times 10^7$  cells/mL. The Cellbag bioreactor requires no cleaning or sterilization, providing easy operation and protection against cross-contamination.

As part of ReadyToProcess platform, the WAVE Bioreactor brings flexibility and speed to upstream and downstream processing of biologicals. The product range comprises WAVE Bioreactor systems, WAVE Mixer™, tubing sealers and fusers, hollow fiber and normal flow filters, prepacked chromatography columns, and ÄKTA™ ready chromatography system with a disposable flow path, as well as the assemblies and connections in between. The platform is scalable from the lab bench to manufacturing.

## WAVE Bioreactor 500/1000 system delivers:

- Convenience: Presterilized, single-use Cellbag bioreactors protect against the risk of cross-contamination, require no cleaning, and involve minimal validation and they are supplied in a ready-to-use format
- Reliability: Cellbag bioreactors, including all fittings and filters, are supplied sterile and ready for use. They are suitable for cGMP commercial production and a biosafety cabinet is not required for inoculation or sampling
- Flexibility: Multiple instrument configurations for suspension, microcarrier, batch, fed-batch, or perfusion culture
- Versatility: The 500/1000 system is capable of handling culture volumes from 50 to 500 L



**Fig 1.** The WAVE Bioreactor 500/1000 system is suitable for culture volumes of 50 to 500 L.

## System descriptions

The WAVE Bioreactor 500/1000 system comprises integral rocking, temperature, weight, and airflow controllers. The self-contained system is designed for use with working culture volumes of between 50 and 500 L in applications such as inoculum scale-up, R & D, process development, commercial production, vaccine production, and antibody manufacture. For additional flexibility, optional modules including DO, CO<sub>2</sub>, O<sub>2</sub>, weight/perfusion, and pH control can be added while builtin Ethernet and MODBUS data ports allow communication with other software.

## Applications

The WAVE Bioreactor system is suitable for use with anchorage-dependent cells in addition to cell suspensions and has applications in:

- Monoclonal antibodies
- Insect cell culture
- Virus production
- Growing pathogens or other high-containment systems
- Inoculum scale-up
- Protein expression
- Primary cell line expansion



## Components

### Touchscreen

The color touchscreen (Fig 2) located on the control panel of the WAVE Bioreactor 500/1000 system enables the setup, control, and viewing of all cell culture parameters while data can be monitored graphically in real time. The main menu provides an overview of key operating conditions and it is the main access screen for all controls. Different control buttons are displayed depending on the options enabled. Pressing the desired button will take you to the respective control screen. The touchscreen is housed in a stainless steel enclosure and can be tilted and rotated for easier viewing.



Fig 2. The touchscreen provides easy access to all control functions.

### Expansion slots

Optional modules such as the dissolved oxygen (DO), O<sub>2</sub>, CO<sub>2</sub>, pH, and dual system controllers can be added to standard WAVE Bioreactor systems to monitor and control additional parameters as your requirements change. These modules plug into the front and back instrument panels (Fig 3) of the base unit and are enabled via the instrument's configuration and calibration functions in order to display the module variables and controls on the color touchscreen. Spare racks are included for future instrument options.



Fig 3. Expansion slots are provided for the installation of optional instrumentation.

### Linear electronic motor

An electric linear motor is used to rock the base units of the WAVE Bioreactor 500/1000 system. Unlike geared motors, this electromagnetic device has only one moving part and provides greater reliability. The linear motor follows a preset and optimal speed and acceleration profile to provide the most effective wave for efficient low-shear mixing.

### Quick-release bag holder

Rapid release Cam-lock levers secure the Cellbag bioreactor in place on the rocking platform allowing bags to be attached and removed in minutes. The holder design ensures that the Cellbag bioreactor is locked in the optimal position for oxygen transfer and mixing.

## Optional components

### pH Monitor

The monitor enables online measurement of pH using a single use electrochemical probe preinstalled in Cellbag bioreactors.

### Dissolved oxygen monitor

The dissolved oxygen (DO) monitor provides amplification, display, and data transmission of DO concentration allowing real-time measurement of DO concentration inside the Cellbag bioreactor. The DO monitor controller was designed for use with miniature fiber optic dissolved oxygen probes (DOOPTROBE), and it can increase the rocking rate automatically to maintain online control of DO.

### O<sub>2</sub>/air mix controller

The O<sub>2</sub>/air mix controller connects to a supply of oxygen (and low pressure N<sub>2</sub> supply if required) to provide O<sub>2</sub>/air concentrations between 0% and 50% O<sub>2</sub>. The instrument controls enriched oxygen levels for insect cell/baculovirus and high culture density applications; it is also useful for maintaining low-oxygen environments for near-anaerobic applications.

### CO<sub>2</sub>/air mix controller

The CO<sub>2</sub>/air mix controller connects to a supply of 100% CO<sub>2</sub> to provide CO<sub>2</sub>/air concentrations between 0% and 15% CO<sub>2</sub>. The instrument is useful for pH control of bicarbonate buffered cell culture media.

### Analog output card

Up to eight channels of analog outputs are available as an option for controlling instrument variables such as rocking speed, weight, airflow, temperature, pH, DO, CO<sub>2</sub>, and O<sub>2</sub> within their preset ranges. The DB25-pin analog output connector is located on the rear panel of the rocker base unit. Two analog output cards are required for dual-configured systems.

### Loadcell

Electronic loadcell modules provide online measurement of Cellbag bioreactor weight and can be used for automated filling and harvesting of media. A built-in pump controller maintains a constant volume for perfusion operations. Loadcell modules are optional factory-installed accessories for WAVE Bioreactor 500/1000 system.

## Technical information and specifications

### WAVE Bioreactor 500/1000 system

Features	Touch panel operator interface
	Direct drive electronic linear motor
	Adjustable rocking rate from 4 to 25 rocks/min with acceleration control
	Adjustable rocking angle from 0.5° to 4° Integral temperature controller with heater Integral weight controller
	Integral airflow controller
	Integral PID controller for automatic temperature, O <sub>2</sub> , CO <sub>2</sub> , and pH adjustment
	Real-time data monitoring
	RS-485 MODBUS communications port
	10Base-T Ethernet communications port Remote alarm contact and printer interface Stainless-steel containment enclosure
Dimensions (L × W × H)	BASE unit: 201 × 124 × 160 cm (79 × 49 × 63 in)
	With KIT500EH: 226 × 124 × 160 cm (89 × 49 × 63 in)
	With KIT1000EH: 226 × 231 × 160 cm (89 × 91 in × 63 in)
Weight (empty)	BASE500/1000EH: 700 kg (1500 lbs)
	With KIT500EH: 925 kg (2000 lbs)
	With KIT1000EH: 1020 kg (2250 lbs)
Utilities	Voltage: 100-120/220-240 VAC
	Frequency: 50/60 Hz
	Maximum current: 15 A
	Power: 12 KVA
Environmental	This equipment is designed for use under the following conditions:
	• Indoor use
	• 5°C to 40°C
	• Up to 80% maximum relative humidity (rh) at 31°C decreasing linearly to 50% rh at 40°C

## Ordering information

Product	Code number
SYSTEM1000EH CO2 O2 DOOPT PH AN	28-4115-59
SYSTEM1000EH DOOPT	28-4115-46
SYSTEM1000EH O2 DOOPT AN	28-4115-60
SYSTEM1000EH CO2 O2 DOOPT PH	28-4115-61
SYSTEM1000EH O2 DOOPT AN	28-4115-62
SYSTEM1000EH CO2 PH	28-4115-63
KIT500EH	28-4115-31
KIT1000EH	28-4115-32

### Related literature

Disposable Cellbag bioreactors for the WAVE Bioreactor system, Data file	28-9511-36
ReadyToProcess connectivity, Data file	29-0138-84
WAVEPOD™ II Integrated Controller, Data file	28-9606-57
WAVE Bioreactor 2/10 and 20/50 systems, Data file	28-9520-58
WAVE Bioreactor 200 system, Data file	29-0237-13



# Xcellerex™ XDR cell culture bioreactor systems

The Xcellerex XDR bioreactor product line offers the benefits of single-use technology and stirred-tank design in a modular, turnkey, bioreactor platform (Fig 1). Designed for scalability and robustness, the XDR bioreactor systems provide the performance and flexibility needed from process development to large-scale biopharmaceutical manufacturing. XDR bioreactor systems can be operated in batch, fed-batch, and perfusion modes.

## XDR bioreactor systems offer the following benefits:

- Scalable and predictable stirred-tank performance up to 2000 L
- Single-use technology eliminates costly and time-consuming cleaning and cleaning validation
- Advanced automation and modular system design to support a variety of installation scenarios
- Process and operational support from staff with extensive manufacturing experience

## System overview

The full line of XDR bioreactor systems are designed and characterized to deliver scalable process equivalence from 4.5 L to 2000 L working volume (Fig 2), in both GMP and non-GMP environments. The system design is based on the same foundational principles as conventional stainless steel bioreactors. Consistent vessel geometries, tight relationship between consumables and equipment features, and a host of accessories combine for an easily scalable, single-use product line. Traditional scaling methodology, based on measures such as shear, tip speed, power per unit volume, kLa, and specific process sensitivities, can be used during scale-up. Technology transfer using the XDR systems is straightforward, minimizing the need for costly and time-consuming process redesign. For enhanced utility across the bioreactor platform, the minimum working volume is as low as 20% of the maximum working volume.



**Fig 1.** Bioreactor system components: XDR vessel with frame-mounted I/O cabinet and X-Station mobile control console.

The bioreactor bag assembly (XDA) is disposed after culture termination and eliminates costly and time-consuming cleaning-in-place (CIP) and steam-in-place (SIP) operations. The XDA bag assembly is prepackaged with a low profile impeller, a variety of sparge components, filters, and tubing, for quick and hassle-free installation. The flexibility of single-use technology enables quick changeover between productions, for efficient equipment utilization. Interconnection of bioreactor bag and equipment is key in achieving the excellent performance that XDR bioreactor systems deliver.

The modularity of the XDR product line stems from three key system components: bioreactor frame, I/O cabinet, and the X-Station mobile control console. The system components



**Fig 2.** The complete range of XDR bioreactor systems are available with maximum working volumes ranging from 10 to 2000 L, from the smallest XDR-10 (described separately in data file 29-0929-27) to the largest XDR-2000 system. Images are representative, some features and accessories may vary depending on the bioreactor configuration.



can be used together for a complete turnkey system with true plug-and-play performance. Alternatively, the components can be used separately and integrated into existing infrastructure, for enhanced flexibility. The jacketed vessel has a consistent design and delivers integrated heating and cooling for efficient temperature control throughout all scales. The versatile I/O cabinet houses all critical process instrumentation such as mass flow controllers, peristaltic pumps, and probe transmitters. This instrumentation can be configured or customized with up to six mass flow controllers as well as up to four internal and two external pumps. The X-Station mobile control console is the heart of the product line's turnkey capability. The power and versatility of the X-Station allow for up to six XDR bioreactor systems to be controlled from one single control unit. This truly modular design creates a process-ready system upon delivery and also supports integration into a user-preferred automation platform.

## XDR system components

### *Well-mixed bioreactor vessel*

Constructed of 304 grade stainless steel (304 SS), the jacketed vessel enables efficient heat transfer and, together with an external temperature control unit (TCU), offers highly accurate temperature control of the cell culture. The bioreactor vessel features load cells for weight measurement and locking casters with leveling feet. Other features include a bioreactor bag tubing manager for convenient positioning and routing of the bag tubing and a high-performance, bottom-mounted, magnetically coupled drive system. Because of the bottom drive, there are no shafts to install from the top of the bioreactor vessel, minimizing ceiling height requirements. To aid in coupling and decoupling of the drive system with the bioreactor bag, motor lifting assistive devices are integral with the two largest bioreactor systems.

The systems are equipped with inlet and exhaust filter holders and vessel sidewall viewing ports. A lower sidewall port opening makes room for a sampling port as well as probes for pH, dissolved oxygen (DO), and temperature. An optional perfusion-specific bag loading door is available to accommodate cell retention devices. The 1000 L and 2000 L systems feature integrated bag loading doors that, along with the semiautomatic bag hoist, simplifies bag insertion without the need for climbing ladders. An efficient exhaust gas filter heater is also included to avoid condensate that could compromise exhaust filter performance.

The complete XDR bioreactor system product portfolio supports operating volumes from 4.5 L up to 2000 L. The smallest process development system is discussed in detail in a separate data file (29-0929-27).

### *Versatile I/O cabinet*

Fabricated in 304 SS, the NEMA4X-rated I/O cabinet houses devices for liquid and gas management as well as pH and DO transmitters. Profibus™ standard is used in device communication and communication to X-Station or other control

systems (e.g., Rockwell, DeltaV™, Honeywell, Siemens, and Mitsubishi systems). Standard XDR gas and liquid management configurations cover the majority of cell culture applications.

### *Liquid management*

The I/O cabinet can be configured with up to four variable-speed peristaltic pumps with ranges to support liquid addition or removal. The pumps can be programmed for fed-batch and perfusion culturing and easily calibrated using Wonderware™ software.

### *Gas management*

Up to six mass flow controllers offer multiple sparging regimes, CO<sub>2</sub> abatement at large scale, and overlay gas addition. The XDR systems include a gas manifold to distribute the various gases to the available bag destinations: sparger or headspace.

### *Measurements of DO and pH*

DO and pH can be measured using conventional polarographic sensors and glass electrodes, respectively. These sensors can be autoclaved prior to use in a specially designed probe sheath. Aseptic insertion into the bioreactor bag is conveniently done using single-use connector technology. Alternatively, an optical DO sensor and a single-use pH probe are available ready for use to minimize start-up time. The flexibility of the system allows the sensor technology to be mixed for use of conventional and single-use technologies simultaneously. All sensors are connected to the I/O panel through transmitters.

### *Measurement of dissolved carbon dioxide*

Conventional, reusable, insertion-type, probe technology is used for monitoring of dissolved carbon dioxide. A dedicated transmitter is available optionally for integration into the I/O panel.

### *Plug-and-play X-Station mobile control console*

X-Station is a stand-alone, mobile control console featuring intuitive process control, data historian, and industrial-quality automation hardware and software (Fig 3). The control system provides real-time data acquisition, enables accurate process control, and offers convenient, real-time trending. X-Station is capable of measuring and controlling up to six XDR bioreactor systems simultaneously.

Inside the 304 SS cover is housed a scalable programmable logic controller (PLC)/programmable automation controller (PAC) and a server-class computer running user interface and data historian software. X-station comes with a 19" touchscreen, industrial, wash down-resistant mouse, a QWERTY keyboard and a built-in uninterruptible power supply (UPS). Profibus and Ethernet communication standards are included for equipment and local area network connectivity.

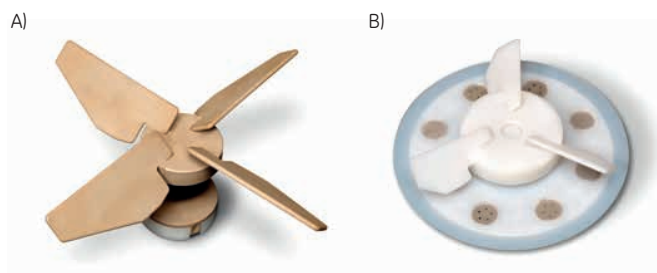


**Fig 3.** The X-Station mobile control console.



## XDA bioprocessing consumables

The XDR bioreactor bag is an essential part of the process performance achieved with XDR bioreactor systems. Constructed with a contact layer of USP class VI-compliant low-density polyethylene (LDPE) plastic, the bioreactor bags are robust to withstand process conditions. All bioreactor bag assemblies incorporate a seal-less, bottom-mounted, impeller/sparger assembly with a centrally positioned integral magnet (Fig 4A). Installed in the bioreactor vessel, the impeller/sparger assembly couples with the magnetic drive head, creating a powerful and robust agitation system with minimal risk of seal leakage. Up to eight sparge elements are included in the impeller/sparge assembly (Fig 4B). Each sparge element may be configured with various porosities, drilled holes, or a combination of both to support both macro- and microsparging. Each of the standard sparge elements have been validated to provide out-of-the-box performance consistent with current cell culture practices.



**Fig 4.** (A) Impeller for XDR-2000 with four pitched blades. (B) Impeller/sparger assembly for XDR-200 with three pitch blades and eight sparge elements.

The XDA bioreactor bags are available in Pro, Development, or Custom formats. Pro-type bags are available for all bioreactor systems and have validated sparge configurations, established based on customer feedback and our own biomanufacturing experience. Development bags are available for XDR-50 and XDR-200 bioreactor systems and differ from the Pro bags primarily in the configuration of the sparge elements. Development bags may be used in a broad array of process development activities where various micro- or macrosparging regimes are evaluated. Custom bioreactor bags can be modified with tubing type and quantity, connection type, filter element, or sparger configuration. Custom bags can also incorporate a CO<sub>2</sub>-removal sparge wand to address processes sensitive for dissolved CO<sub>2</sub>.

All bioreactor bags for cell culture include a magnetically coupled, M40e 40°, pitched-blade impeller with ceramic bearings. Ceramic bearings, originally developed for aggressive mixing applications and fermentation, provide excellent overall performance. The medical-grade ceramic bearings meet the relevant industry requirements for leachables, extractables, and particulates according to the bag validation guide (for more information, please contact your sales representative). Special impellers are available for microcarrier applications.

Additional bag components include tubing with a combination of weldable sections and aseptic connectors for liquid addition/removal, a disposable pressure sensor, and filters for exhaust, sparge, and overlay/headspace gas. XDA bioreactor bags support both insertion-type reusable probes and single-use probes. Probe configurations include reusable probes only, single-use probes only, or mixed reusable and single-use probes. For details on probes, see section “Measurement of DO and pH”.

To assist in efficient bioreactor operation, a number of supplementary XDA bag-related accessories are optionally available. Like the main bioreactor bags, these accessories are tightly coupled to the equipment design to be adaptable to varied process requirements and to support operational efficiency. XDA accessories include seal-and-store sample manifolds, foam traps, X-Connect tubing sets, and exhaust filter tubing sets.

## Qualification support

The XDR bioreactor systems are designed for use in environments that require 21 CFR Part 11 and Good Automated Manufacturing Practice (GAMP) 5. The systems are delivered with an operating manual, system specification, drawings to support qualification, and major component documentation. Industry standard installation and operation qualification (IQ/OQ) packages are available as an option.

## Applications

XDR cell culture bioreactor systems have successfully been used to cultivate a wide range of cell types and organisms including CHO cells, Vero cells, and MDCK cells. In addition, a fermentor system is available for microbial applications including *E. coli*, *Pseudomonas spp.*, and yeast (see data file 29-0929-29). XDR bioreactor systems can be operated in batch, fed-batch, and perfusion (or chemostat) modes. Bioreactor bag design can be process-dependent, requiring customization for proper use and performance.

## System specifications

Standard cell culture specifications are listed in Table 1.

## Ordering information

Related literature	Code number
Xcellerex XDR-10 cell culture bioreactor system, data file	29-0929-27
Xcellerex XDR-50 MO fermentor system, data file	29-0929-29
Xcellerex XDUO Quad Mixing System, data file	29-0483-66
Xcellerex XDM Quad Mixing System, data file	29-0483-67

For more information on the bioreactor systems, please contact your local sales representative.

**Table 1.** Specifications of standard configuration XDR cell culture bioreactor systems

Specification	XDR-50*	XDR-200	XDR-500	XDR-1000	XDR-2000
Max. working volume (L)	50	200	500	1000	2000
Min. working volume (L)	22	40	100	200	400
Volume turn-down ratio	2.2:1	5:1	5:1	5:1	5:1
Aspect ratio (H/D)	1.5:1	1.5:1	1.5:1	1.5:1	1.5:1
Vessel	Jacketed 304 SS				
Filter heater assembly	1	1	1	1	1
Additional filter heater assemblies (optional)	1	1	1	1	1
Bag hoist	Not applicable	Not applicable	Not applicable	Semi-automatic	Semi-automatic
Impeller, M40e pitched-blade type	3 blades at 40° pitch				4 blades at 40° pitch
Standard bag assembly	Development: 29-0410-65 Pro: 29-0410-60	Development: 29-0410-66 Pro: 29-0410-61	Pro: 29-0410-62	Pro: 29-0410-63	Pro: 29-0410-64
Custom bag assembly	Available on request				

**Process instrumentation**

pH probes†	1 (optional second probe)	2	2	2	2
DO probes†	1 (optional second probe)	2	2	2	2
MFC§ (standard)	4	4	4	5	5
Additional MFC‡ (optional)	2	2	2	1	1
Internal pumps (standard)	3 (2× low flow, 1× high flow)	3 (2× low flow, 1× high flow)	3 (2× low flow, 1× high flow)	4 (2× low flow, 2× high flow)	4 (2× low flow, 2× high flow)
Additional internal pumps (optional)	1	1	1	Not applicable	Not applicable
External pumps (optional)§	2	2	2	2	2
Temperature control unit¶ (heating, kW/cooling, HP)	3/1	9/1.5	9/1.5	9/1.5	9/1.5
Load cells	3	4	4	4	4

**X-Station control unit**

Interfaces	19"/52.4 mm touchscreen, wash-down compatible keyboard and mouse; includes built-in UPS				
Hardware	Rockwell/Allen Bradley				
Operator Interface	Wonderware HMI††				
HMI†† Data Historian	Wonderware				
Compliance	Built to GAMP5 standards/ 21 CFR Part 11 compliant††				

\* For specifications of XDR-50 MO fermentation system, please see data file 29-0929-29.

† Single-use or reusable probes

‡ Thermal mass flow controllers

§ External pumps are Profibus devices and may be Watson-Marlow™ 520, 620, or 720 series depending on required flow rate.

¶ Optional standalone heating/cooling TCU listed. Other TCU configurations available, including units that use facility chilled water/glycol.

†† Human-machine interface

‡‡ Customer will need to implement appropriate operating procedures to be fully 21 CFR part 11 compliant. The XDR is built to support this two-part compliance.

**Note!** For specifications of XDR-10 bioreactor system, please see data file 29-0929-27.

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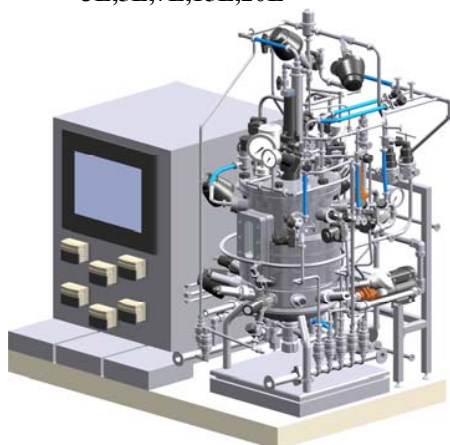
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## TECHNISERV 3L GLASS TO 25,000L PRODUCTION BIOREACTORS POWERED BY CONTROL PLATFORMS FROM EMERSON, SIEMENS OR ALLEN BRADLEY.

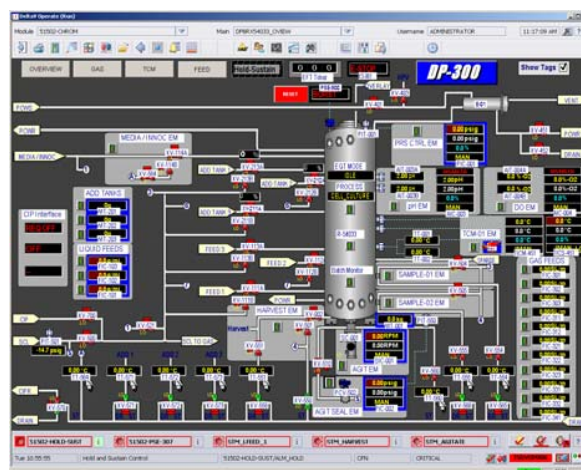


**SCAL-TECH® MINI**  
Glass Laboratory Scale  
3L,5L,7L,15L,20L

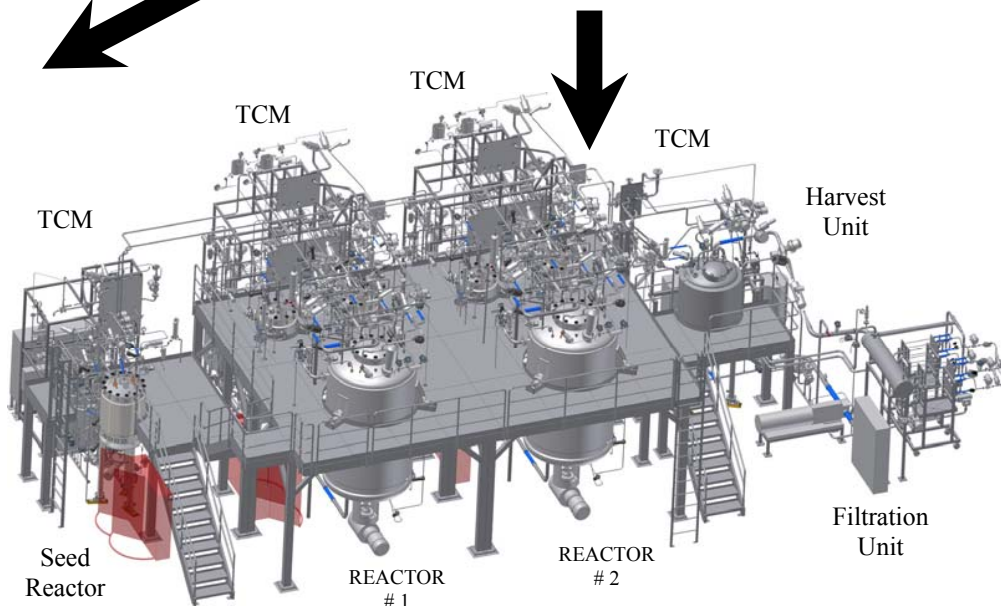


**SCAL-TECH® BENCH**  
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Established in 1982, the Techniserv Automation & Integration Group can supply the Emerson DeltaV, Siemens DCS and/or Allen Bradley Control Logics based control systems on Bioreactors from our 3L glass to 25,000L production systems with Identical look, feel, and recipe function on any size reactor.

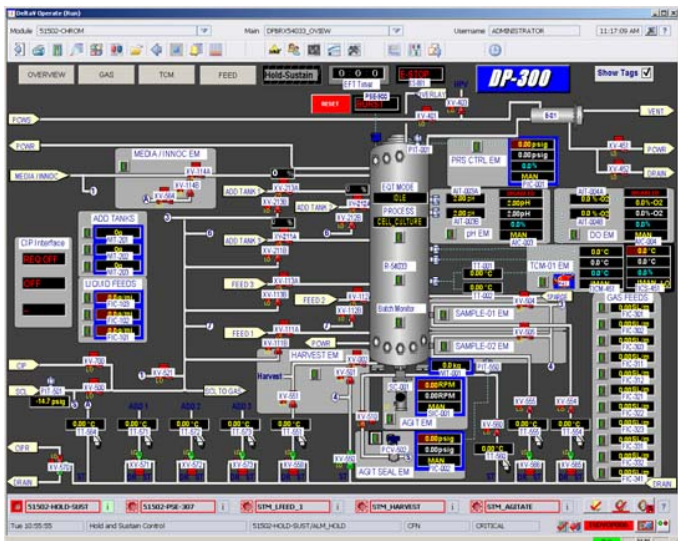


30L S/S Through 200L S/S



**SCAL-TECH® Pro Suites**  
200L S/S Through 25,000L S/S





# TECHNISERV, INC.

## Company Profile:

The Techniserv, Inc. *Bioreactor and Fermentation* and *Process Equipment* divisions are focused on providing both standard and custom process systems to the Pharmaceutical, Biotech and related high purity industries, and have supplied many large modular systems to these companies. Our capabilities in design, fabrication, software development and validation allow delivery of fully Turnkey integrated systems. Techniserv's client list encompasses the majority of the largest US and International Bio-Pharmaceutical manufacturers.

### Featuring Emerson Delta V Operating System

division also provides alternate customized control solutions utilizing conventional control platforms including Allen Bradley PLC's, Emerson Delta-V DCS and Siemens PCS-7 DCS. Techniserv has developed many SCADA systems utilizing packages such as Intellution, Rockwell RSView, Wonderware and more.

Techniserv, Inc. employs ten (10) electrical and automation engineers in the *Control System Integration* division, eleven (11) mechanical and process engineers in the *Bioreactor and Fermentation* and *Process Equipment* divisions, and multiple dedicated project managers and validation specialists.

Techniserv's off the self standard bioreactor and fermentor systems can incorporate the Emerson Delta V control package. For the first time, Techniserv can offer a Delta V based DCS control platform that seamlessly supports 3L Glass through 25,000L production reactors.

Established in 1982, Techniserv's *Control System Integration*

- Control Platforms:  
Allen Bradley PLC  
Emerson DeltaV DCS  
Siemens PCS-7 DCS

- Migration from Lab to Production
- FDA 21CFR Part 11 Compliant
- Utilizes Bused I/O Technologies
- Supports PAT



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Sharpville Container is known for high quality fabrication in stainless steel and exotic alloys. Our vessels, whether they are process or transport, ASME, UN, or Heat Transfer are manufactured for the Food, Chemical, Beverage, Petro-Chemical and Pharmaceutical markets. The size of vessels that we manufacture range from 1 gallon to 25,000 gallons. With our standard line and custom design expertise, we are capable of meeting or exceeding your fabrication requirements. Our reputation for quality and service continues to exceed our customers' expectations.



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## STORAGE TANKS

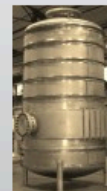


### TYPES

1. Kettles
2. Surge Tanks
3. Cone Bottom
4. Vertical or Horizontal

### OPTIONS

1. Foundation or Leg Options
2. Skid Mounted
3. Variety of finish and polis options
4. Aseptic Storage and Cleaning





# Culturefuge 100

## Solids ejecting centrifuge

Many new biological products are derived from fragile organisms. Although relatively easy to separate the trick is accomplishing the separation in a gentle manner without destroying the shear sensitive cell wall membranes that isolate the complex intracellular proteins from the extracellular liquid. If this can be avoided, downstream purification of the target proteins becomes much easier.

### Applications

The machine is designed for clarification duty. Especially when clarifying liquids from shear sensitive particles. Applications that requires low oxygen pick-up can also take advantage of the hermetic features this machine offers.

### Standard design

The machine consists of a frame that has a horizontal drive shaft, worm gear, lubricating oil bath and hollow vertical bowl spindle in the lower part. The bowl is mounted on top of the spindle, inside the space formed by the upper part of the frame, the ring solids cover, the collecting cover, and the frame hood. The liquid discharge system also rests on this structure. All parts in contact with the process liquid are made of stainless steel. The bowl is of the solids-ejecting disc type with an automatic hydraulic operating system for discharging. It is a so-called timer triggered partial discharge system, meaning that only part of the bowl content is emptied during pre-set discharge intervals. The discharge takes place at full speed without any interruption of the feed. The centrifuge is available with main connections as sanitary flanges and all other utility connections clamp type. The electric motor is of standard type and has a built-in variable frequency drive. The design conforms with a number of EC directives, and machine is made in accordance with the general directives for machinery. Finally, the centrifuge is equipped with nozzles for flushing of the bowl top, the bowl bottom and the cyclone.

### Standard equipment

Each Culturefuge 100 centrifuge comes with control unit,



Fig. 1 Culturefuge 100 centrifuge

electric motor, in- and outlet connections, spare parts kit and set of tools.

### Material data

Bowl body, hood and lock ring	s.s. 1.4462 UNS S31803
Solids cover and frame hood	s.s. ASME SA-240 UNS 31603
Cyclone	s.s. ASME SA-240 UNS 31603
Bottom frame	Cast grey iron
In and outlet	s.s. mostly 1.4401 UNS 31600
Gaskets and O-rings	EPDM rubber (FDA approved)



## Operating principles

The feed is introduced to the rotating centrifuge bowl (fig 2) from the bottom through a hollow spindle (1), and is accelerated in a distributor (2) before entering the disc stack (3), where the separation takes place. The separated liquid phase leaves through the liquid outlet (4) at the top of the bowl. The collected solids in the solid space (5) are intermittently discharged from the periphery of the bowl. During normal production the operating water keeps the sliding bowl bottom (6) closed against the bowl hood (7). During discharge the sliding bowl bottom drops for a short time (less than a second) and the solids are ejected through the discharge ports (8). The high velocity of the ejected solids is reduced in the cyclone.

## Available models

The Culturefuge 100 centrifuge is available in pressure vessel designs according to ASME or to PED. In addition, different surface finish executions are available:

Bowl spindle	Ra 0.8
Bowl spindle	Ra 0.5 and electropolished
Machine top part	Inside: Ra 0.8, Outlet cover: Ra 0.5 and electropolished
Machine top part	Inside: Ra 0.8, Outlet cover: Ra 0.8
Machine top part	Inside: Ra 1.2, Outlet cover: Ra 1.2
Separator bowl	Inside: Ra 0.5 and electropolished, Outside: Ra 0.8
Separator bowl	Inside: Ra 0.8, Outside: Ra 0.8
Separator bowl	Inside: Ra 1.2, Outside: Ra 1.2

## Dimensions (approximate)

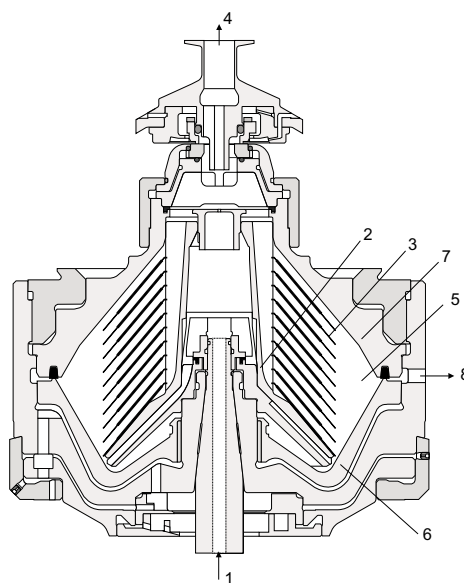
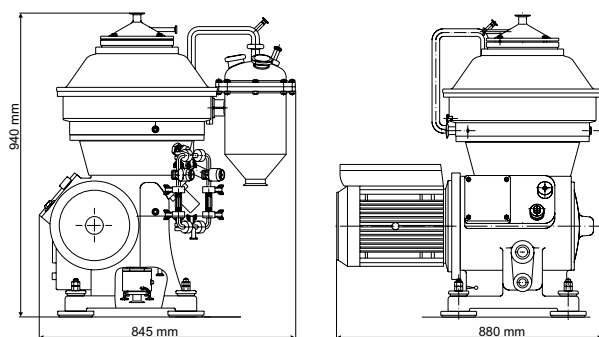


Fig. 2 Typical bowl for a hermetic solids-ejecting centrifuge. The details illustrated do not necessarily correspond to the centrifuge

## Technical specification

Hydraulic capacity	max. 2.0 m <sup>3</sup> /h <sup>1)</sup>
G-force	max. 12200 g
Bowl speed	max. 9650 rpm
Motor power installed	7.5 kW
Sound pressure	74 dB(A) <sup>2)</sup>
Overhead hoist lifting capacity	min. 100 kg

<sup>1)</sup> Actual capacity depends on feed material and separation demands

<sup>2)</sup> In compliance to EN ISO 4871

## Utilities consumption

Electric power	5.5 kW
Operating water	0.3 l/discharge
Cyclone flush	0 - 8 l/discharge
Cooling for seals	max. 300 l/h
Flushing above the bowl	0 - 1 l/discharge
Flushing under the bowl	0 - 1 l/discharge
Steam per sterilization cycle	5 - 10 kg

## Shipping data (approximate)

Centrifuge with bowl with motor	450 kg
Gross weight	600 kg
Volume	1.0 m <sup>3</sup>

## How to contact Alfa Laval

Contact details for all countries are continually updated on our website. Please visit [www.alfalaval.com](http://www.alfalaval.com) to access the information direct.

# Chromaflow columns

Chromaflow™ columns are a family of convenient to use, process-scale columns. A patented nozzle in the top and bottom of the column allows packing, unpacking, and cleaning when fully assembled, that is with the lid in place. Chromaflow columns simplify chromatographic procedures and offer:

- convenience
- saving of labor
- reproducibility
- contained packing
- scalability

## General column description

Chromaflow low-pressure columns (Fig 1) are available in a choice of dimensions and materials. The complete range offers inner diameters (i.d.) from 300 to 2000 mm (Table 1), with column tubes manufactured from cast acrylic (Fig 1). All dimensions are available with variable bed heights, providing a wide variety of bed volumes. All columns are pressure rated for operation at 3 bar.

Chromaflow columns incorporate a patented, pack-in-place nozzle (Fig 2) through which process liquids enter and exit. Manual or automated versions of the nozzles are available. The automated nozzle is controlled from the packing station or the nozzle control unit. The nozzle has three positions to facilitate the different aspects of column operation: packing, operation, unpacking and cleaning. In addition to this pack-in-place functionality, the nozzle also contains the process liquid flow path to provide a consolidated solution to the process stream handling.

Bed supports are available in 316L stainless steel or polyethylene. The multilayer, woven stainless steel bed supports have very high chemical resistance and longevity for use in applications where salt concentrations are low and pH is above 5. Polyethylene bed supports are recommended for applications with low pH and high salt concentrations. All other wetted parts in columns with polyethylene bed supports are manufactured from plastic or noncorrodible materials for use in low pH /high salt applications.



Fig 1. A Chromaflow column, 2000 mm i.d.



Fig 2. The Chromaflow nozzle that enables packing in place in a fully assembled column (GE Healthcare patent).



The construction materials include 316L stainless steel, acrylic, polypropylene, polyethylene, PEEK 450 G, EPDM rubber and FEP encapsulated silicone. These materials have high chemical resistance to the liquids typically used in process chromatography (Table 2). Furthermore, all polymeric materials are approved according to USP class VI tests for toxicity.

As an option, a dedicated packing station is available for Chromaflow columns. The packing station speeds up the packing procedure by eliminating the more time-consuming, manual maneuvers (Fig 3).

Comprehensive documentation is delivered with each column and includes a User manual, a Maintenance manual, assembly drawings, a full spare part list, materials certificates etc.

A Validation Support File containing information on column component composition, materials of construction and toxicity studies is also available.



**Fig 3.** Packing Chromaflow columns with the dedicated packing station is convenient and simple.

## Convenient and labor saving

Once the column is assembled and the lid in place, no lifting gear is required for packing, operation, unpacking or cleaning-in-place (CIP). This means that a single operator can perform all column operations, thereby reducing labor costs and increasing convenience in large-scale operations.

## Reproducibility

Packing with the lid in place allows the packing parameters to be easily set and fixed. Manual operation is minimized and standard operating procedures can be followed, helping to give reproducible column packing and results.

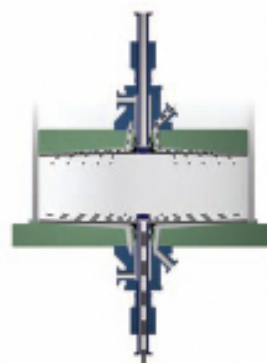
## Contained packing

Improved safety is another advantage of the Chromaflow column concept. Because all the column operations are performed in a "closed system" environment, there is less risk of the operator coming into contact with hazardous chemicals and of the target product being exposed to contamination. In this way, overall safety and hygienic operation are improved.

## Principle of operation

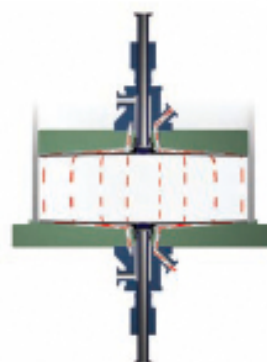
The column has a three-position nozzle located in the center of the top and bottom bed support. These three positions enable packing, unpacking, operation and cleaning to be performed without any adjustments to the assembled column, that is the lid remains in place.

Flow profiles from the two nozzles are identical. Packing direction will depend on the characteristics of the media and packing method used. The three positions are illustrated in Figure 4.



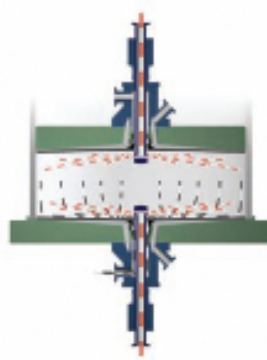
### Packing position

The bottom nozzle is extended part of the way (mid position) into the column. The top nozzle is fully retracted. Slurry enters the column via the bottom nozzle and excess liquid exits via the top mobile phase outlet. After packing, the slurry lines are isolated from the mobile phase and can be cleaned independently from the rest of the column.



### Running position

The bottom and top nozzles are retracted. Mobile phase enters the column directly into an annulus, immediately behind the bed support. The annulus is cut through at an angle to ensure that linear flow is kept constant during distribution of the mobile phase across the bed.



### Unpacking position

In this position, both bottom and top nozzles are fully extended into the column thereby exposing a third passage through which medium leaves the column.

Cleaning solution can be pumped through the nozzles and sprayed into the column. In this way the column is easily and effectively cleaned without exposing the interior or the medium to the environment, and without dismantling the column.

**Fig 4.** The three positions of the Chromaflow nozzle showing packing from the top.



## Scalability

Chromaflow columns are available in a wide range of dimensions, all designed and constructed around the same design principle. Standard range columns come in dimensions from 400 to 1000 mm, for more information about columns and dimensions, see Ordering information. Scaling up a chromatographic process from small to larger diameters is easily performed with maintained reproducibility, safety and convenience.

## Column dimensions

A selection of Chromaflow columns in the range 400 to 2000 mm i.d. are presented in Table 1. The adapter stroke length is a standard 200 mm. Variable bed heights are available in the ranges 100–300 mm, 200–400 mm and 300–500 mm.

## Chromaflow 400 SFP columns

Chromaflow 400 SFP (small flow path) columns are specially designed for low-flow applications. The dimensions in the mobile phase have been optimized to reduce dead volumes to a minimum and the area behind the nozzle tip has also been reduced.

## Column materials and their chemical resistance

Table 2 lists the major components of Chromaflow columns in contact with process fluids (wetted parts) and Table 3 lists the chemical resistance of materials using data compiled from several published sources. It is important to note that columns with stainless steel bed supports and other stainless steel wetted components must be appropriately maintained when exposed to NaCl. Since salt can be corrosive to stainless steel over time, it is recommended that residual salt is removed by rinsing columns with at least five column volumes (CV) of clean water.

**Table 1.** Weights, volumes and dimensions for variable bed height Chromaflow columns

Description	Max operating pressure (bar)	Volume (l)	Column overall height (mm)	Weight, dry (kg)	Footprint (mm x mm)
Chromaflow column 400/100-300*	3	12.6–37.8	1568	230	700 x 700
Chromaflow column 600/100-300	3	28.3–84.9	1568	375	800 x 800
Chromaflow column 800/100-300	3	50.3–150.9	1572	610	1000 x 1000
Chromaflow column 1000/100-300	3	78.5–235.5	1573	930	1200 x 1200
Chromaflow column 1200/100-300	3	113.1–339.3			
Chromaflow column 1400/100-300	3	153.9–461.7			
Chromaflow column 1600/100-300	3	201.1–603.3			
Chromaflow column 1800/100-300	3	254.5–763.5			
Chromaflow column 2000/100-300	3	314.2–942.6			

\* The first figure in the column name indicates the inner diameter and the second figure indicates stroke length.

**Table 2.** Major components and their composition

Component	Material	In contact with process stream
Column tube	Acrylic or stainless steel 316L	Yes
Column lids	Stainless steel 316L	No
Distributor	Polypropylene	Yes
Bed support	Stainless steel 316L or polyethylene	Yes
Chromaflow nozzle	Polypropylene, stainless steel 316L, PEEK 450 G	Yes
Seals	EPDM or FEP encapsulated silicone	Yes
Stand	Stainless steel 316L	No

EPDM = ethylene propylene diene, FEP = fluoroethylenepropene, PEEK = polyetherether ketone

**Table 3.** Chemical resistance of materials used in Chromaflow columns (60 days)

Chemical	Acrylic	SS 316L	EPDM	FEP	PEEK 450 G	PE	PP
Acetic acid 1.7 M	+	+	+	+	+	+	+
EtOH 20% <sup>1</sup>	+	+	+	+	+	+	+
EtOH 40%	-	+	+	+	+	(+)	+
Ethylene glycol 50%	+	+	+	+	+	+	+
Formaldehyde 1.7 M	+	+	+	+	+	+	+
Formic acid 10%	(+)	+	+	+	+	+	+
Glycerol 100%	+	+	+	+	+	+	+
Hydrochloric acid 0.1 M	+	-	+	+	+	+	+
Isopropyl alcohol 30%	-	+	+	+	+	(+)	+
Nitric acid 0.1 M	+	+	+	+	+	(+)	+
Phosphoric acid 25%	+	(+)	+	+	+	+	+
Sodium chloride 0.5 M	+	+ <sup>2</sup>	+	+	+	+	+
Sodium hydroxide 2 M <sup>3</sup>	+	+	+	+	+	+	+
Trifluoroacetic acid 0.1%	(+)	+	+	+	+	+	+
Triton™ X-100 100%	+	+	+	+	+	+	+
Urea 8 M	+	+	+	+	+	+	+

+ Resistant (-) Limited resistance - Not recommended

<sup>1</sup> Do not expose acrylic to concentrations of ethanol greater than 20%. Do not exceed the following parameters during storage: 5 yr, 23°C, 0.5 bar g.

<sup>2</sup> NaCl can cause corrosion on stainless steel at pH <5. Do not use NaCl in storage solutions. Rinse with at least 5 CV of clean water after use with NaCl.

<sup>3</sup> Maximum exposure 4 h.

SS=stainless steel, EPDM=ethylene propylene diene, FEP=fluoroethene propene, PEEK=polyetherether ketone, PE=polyethylene, PP=polypropylene.

## Sanitizing Chromaflow columns

The design of Chromaflow columns facilitates cleaning-in-place. Below is a recommended cleaning protocol suitable for most applications.

1. Circulate 1.5 CV of 20% acetic acid at a low flow velocity (60 cm/h) for 15 min, upward flow. Then reverse the flow for 15 min.
2. Repeat this procedure with 1.0 M NaOH.
3. Following step 2, slowly circulate 1.0 M NaOH in the column for 60 min.
4. Re-equilibrate the column with a storing or starting buffer.

## Chromaflow Packing stations

Chromaflow Packing stations make column priming and packing a simple operation, reducing the operator's time to a minimum. The packing stations consist of a control panel with pumps and valves fitted underneath (Fig 5). Valves and diaphragm pumps are actuated pneumatically from the control panel. As they are brought into operation indicators on the control panel display the relevant flow paths. For operation, packing stations only require a supply of compressed air. To select an appropriate packing station for your column and media, refer to Tables 4 and 5.



Fig 5. Chromaflow Packing station Pack 100.

Table 4. Specifications of Chromaflow packing stations

Designation*	Pump	Pump flow capacity (l/min)	Req. air supply (m <sup>3</sup> /min)	Inlet piping/outlet i.d. (mm)	TC connections (mm)	Weight, dry (kg)	Size W x H x D (mm)
Pack 50	Tapflo™ T53	10–50	0.5	22.1/22.1	50.5	115	810 × 1175 × 715
Pack 100	Tapflo T103	30–100	1.0	34.8/22.1	50.5	130	810 × 1175 × 715

\* Packing stations, Pack 200 and Pack 400 with pump flow capacities of 60 to 200 l/min and 100 to 400 l/min are available as custom orders.

Table 5. Approximate packing flow rates for different media at two different bed heights

Column diameter (mm)		400				600				800				1000			
Bed height (mm)		150		300		150		300		150		300		150		300	
Flow		cm/h	l/min	cm/h	l/min	cm/h	l/min	cm/h	l/min	cm/h	l/min	cm/h	l/min	cm/h	l/min	cm/h	l/min
Sephacore™																	
Fast Flow media		500	11	250	5.5	500	24	250	12	500	42	250	21	500	66	250	33
Sephacore																	
Big Beads media		1600	34	1200	25	1600	75	1200	57	1600	134	1200	101	1600	209	1200	157

## What else do I need?

### The column

The columns are supplied ready for use and are equipped with adjustable feet. Castors can be ordered separately for columns up to 1000 mm in diameter.

### Isolating the column after packing

We recommend using sanitary stainless steel valves (of the appropriate inner diameter) on the mobile phase to prevent contamination of the packed bed. For storage purposes, blind flanges with a clamp and gasket can be used to seal off the column.

### Connecting the column to your system and packing station

Clamps and gaskets of suitable size are required to connect the sanitary flanged inlet/outlet to either valves or tubing of the same type. Preflanged tubing is also available.

### Assembly or disassembly of the column

An adequate sized wrench is needed for assembly or disassembly of the column. A hoist is needed to remove the adapter or top lid from the column.

### Spare parts to keep on site

It is recommended that nozzle seals, column seals, and column bed support kits are kept as spare parts.

### Useful accessories

**Safety valve:** Precalibrated valve which releases pressure if the calibrated value is exceeded. Recommended to install on the mobile phase inlet if no other pressure sensor is included in the chromatography system. The T-junction, clamps and gaskets must be ordered separately.

**Pressure sensor:** The sensor is installed inline, preferably on the mobile phase inlet. Clamps and gaskets have to be ordered separately.



## Ordering information

### Columns

Chromaflo columns with acrylic tubes	Bed support 10 mm SS sinter	Bed support 20 mm SS sinter	Bed support 20 mm PE sinter
<i>I.d. 400 mm Man. nozzle</i>			
Stroke length 100-300	18-1150-40	18-1159-40	18-1161-40
Stroke length 200-400	18-1157-42	18-1159-42	18-1161-42
Stroke length 300-500	18-1157-44	18-1159-44	18-1161-44
<i>I.d. 400 mm Auto. nozzle</i>			
Stroke length 100-300	18-1157-41	18-1159-41	18-1161-41
Stroke length 200-400	18-1157-43	18-1159-43	18-1161-43
Stroke length 300-500	18-1157-45	18-1159-45	18-1161-45
<i>I.d. 400 mm SFP* Man. nozzle</i>			
Stroke length 100-300	18-1170-53	18-1176-12	11-0011-85
Stroke length 200-400	11-0011-80	11-0011-83	11-0011-86
Stroke length 300-500	11-0011-82	11-0011-84	11-0011-87
<i>I.d. 400 mm SFP Auto. nozzle</i>			
Stroke length 100-300	11-0011-89	11-0011-91	11-0011-94
Stroke length 200-400	11-0011-88	11-0011-92	11-0011-95
Stroke length 300-500	11-0011-90	11-0011-93	11-0011-96
<i>I.d. 600 mm Man. nozzle</i>			
Stroke length 100-300	18-1150-60	18-1159-60	18-1161-60
Stroke length 200-400	18-1157-62	18-1159-62	18-1161-62
Stroke length 300-500	18-1157-64	18-1159-64	18-1161-64
<i>I.d. 600 mm Auto. nozzle</i>			
Stroke length 100-300	18-1157-61	18-1159-61	18-1161-61
Stroke length 200-400	18-1157-63	18-1159-63	18-1161-63
Stroke length 300-500	18-1157-65	18-1159-65	18-1161-65
<i>I.d. 800 mm Man. nozzle</i>			
Stroke length 100-300	18-1150-80	18-1159-80	18-1161-80
Stroke length 200-400	18-1157-82	18-1159-82	18-1161-82
Stroke length 300-500	18-1157-84	18-1159-84	18-1161-84
<i>I.d. 800 mm Auto. nozzle</i>			
Stroke length 100-300	18-1157-81	18-1159-81	18-1161-81
Stroke length 200-400	18-1157-83	18-1159-83	18-1161-83
Stroke length 300-500	18-1157-85	18-1159-85	18-1161-85
<i>I.d. 1000 mm Man. nozzle</i>			
Stroke length 100-300	18-1150-10	18-1160-10	18-1162-10
Stroke length 200-400	18-1158-12	18-1160-12	18-1162-12
Stroke length 300-500	18-1158-14	18-1160-14	18-1162-14
<i>I.d. 1000 mm Auto. nozzle</i>			
Stroke length 100-300	18-1158-11	18-1160-11	18-1162-11
Stroke length 200-400	18-1158-13	18-1160-13	18-1162-13
Stroke length 300-500	18-1158-15	18-1160-15	18-1162-15

For column specifications other than listed in the table, please contact your local GE Healthcare representative.

\* SFP = Small Flow Path on mobile phase, only available on 400 mm i.d. columns.

[www.gehealthcare.com/protein-purification-bioprocess](http://www.gehealthcare.com/protein-purification-bioprocess)

GE Healthcare Bio-Sciences AB

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Pall Corporation

Contact Us: [www.pall.com/contact](http://www.pall.com/contact)

## Cadence™ Single-Pass Tangential Flow Filtration Modules and Systems

### Description

#### Eliminate the Recirculation Loop and Streamline Operations to Optimize Downstream Processing

Cadence Single-Pass TFF ( SPTFF ) is a patented breakthrough technology that allows direct flow-through concentration with no recirculation of product. SPTFF enables high concentration of shear sensitive proteins and antibodies >160 grams/liter. What makes this possible is a unique flow path design and staging of cassettes in a serial flow.

See the Cadence™ system in action, [watch the video](#).

#### Single-pass TFF patent numbers:

- ▶ US patent number 7.682.511 B2
- ▶ US patent number 7.384.549 B2
- ▶ US patent number 7.967.987 B2
- ▶ US patent number 8.157.999

#### This exciting new technology leads to economic and practical benefits, including:

- ▶ Increased yield of product due to improved recovery capability and lower hold-up volume.
- ▶ Increased final product concentration due to high concentration factor capability.
- ▶ Elimination of product damage or aggregation due to reduced residence time and shear exposure.
- ▶ Ability to couple the concentration of product before or after other downstream process steps, consequently optimizing other steps and reducing in-process pool tank volumes.
- ▶ Available in single use format.

Cadence single-pass TFF modules and systems can be easily integrated with other downstream processes such as chromatography or virus removal.

Using Cadence modules with Delta membrane, the innovative benefits of Single-Pass TFF technology are enhanced by the proven high flux, high selectivity, and low protein binding attributes of Delta regenerated cellulose membrane cassettes.

#### Optimize Economic and Operational Benefits

- ▶ **Enhance downstream processing to increase capacity and reduce costs** – Cadence systems can eliminate or reduce intermediate storage tanks and associated cleaning of tanks when using the systems for in-process volume reduction before or after existing steps.
- ▶ **Increase yield and achieve high product recovery > 98 %** – The single-pass TFF process collects most of the process volume during the process run, leaving only a small percentage of the product in the module at the end of the run. This small percentage of product can be easily recovered due to the low hold-up volume in the system.
- ▶ **Enable high concentration factors > 20X** – High concentration factors are achieved due to the staging of cassettes in series, which results in high conversions of feed-to-permeate in one flow path. This is accomplished by optimizing the module staging configuration based on cassette performance for a particular application.
- ▶ **Optimize processing of highly shear-sensitive products** – Processing results in only one pass through the pump and cassette, reducing shear exposure. For products sensitive to pumping, the pump can be completely eliminated by using pressurized vessels to flow the process fluid through the module. Further benefits are achieved by eliminating any mixing or foaming issues associated with the feed tank.
- ▶ **Use smaller, more compact systems** – Cadence systems require less pump capacity, allowing smaller piping or tubing diameters for a more compact system design with lower working and hold-up volumes.
- ▶ **Simplify installation and use** – Cadence modules are supplied preassembled. The modules' feed, retentate and permeate ports are easily connected to clearly-marked system ports.



## Product Platform

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Innovative single-pass TFF processing takes place within Cadence modules. Cadence modules incorporate Pall's proven T-Series cassettes with Delta membrane, and are available in different sizes to accommodate various processing volumes (see Ordering Information). Cadence modules are designed for use with a Pall Cadence single-pass TFF system. (Contact Pall for information on a Cadence system to meet your application requirements.)

## Cadence Modules Incorporate High Performance T-Series Cassettes with Delta Membrane

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Delta regenerated cellulose membrane offers high flux and selectivity. The membrane has been specifically developed to minimize protein binding to the surface and interstitial structure of the membrane. Inherently hydrophilic, this membrane shows low protein adsorption properties and is optimal for processes involving very hydrophilic proteins. Delta regenerated cellulose membrane shows low fouling characteristics, allowing consistent performance or flux from run to run. It is easy to clean and typically recovers normalized water permeability (NWP) by using 0.1 N NaOH.

## Cadence T01 Module Installed in Holder



## Application

Cadence single-pass TFF modules and systems are used to concentrate biomolecules. They also can be used for salt-level reduction by concentrating followed by in-line dilution. Volume capacities range from several liters to thousands of liters. Single-pass TFF may be used to perform various steps in a wide range of applications in the biopharmaceutical industry, including:

- ▶ In-process volume reduction
- ▶ Desalting protein solutions
- ▶ Purification and recovery of antibodies or recombinant proteins
- ▶ Preparation of samples (concentrating, desalting) before or after column chromatography

## Single-Use Applications

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Depending on the application, Cadence modules may also be applied for single use. When applied for single use, Cadence modules are used with a specially-designed Cadence system. Contact Pall for more information on single-use systems.

### Application Note: Cadence™ Systems Employ New Single-Pass TFF Technology to Simplify Processes and Lower Costs

- ▶ The commercialization of a revolutionary TFF technology by Pall Life Sciences presents opportunities to simplify biopharmaceutical process design and lower capital and operational costs. The new technology, single-pass TFF, is brought to life through Pall's Cadence systems and modules. This Application Note introduces the design innovations of Cadence single-pass TFF systems to biopharmaceutical researchers and manufacturers.

**Protein Concentration with Single-Pass Tangential Flow Filtration (SPTFF):** This peer-reviewed journal article on the Cadence Single-Pass Tangential Flow Filtration (SPTFF) technology has been published in the Journal of Membrane Science (Volume 384, Issues 1-2, 2011, pp 82-88). The article highlights the novel SPTFF process including:

- ▶ SPTFF process is continuous, produces high concentration factors, significantly higher conversion in one pass through flow rating control and eliminates the need for the conventional recirculation loop.
- ▶ Elimination of the conventional recirculation loop minimizes aggregation problems and requires no mixing, minimizes shear exposure and also allows the SPTFF step to be coupled with other downstream process steps.
- ▶ Additional SPTFF benefits over conventional TFF include lower system hold-up volumes, higher recoveries, and lower flush volume requirements.
- ▶ Applications that can leverage SPTFF technology include but are not limited to in-process volume reduction, inline salt reduction, high-concentration formulations, and processing of fragile biomolecules.

## Specifications

The operating conditions and module configurations for any single-pass TFF process must be established by performing trials and analyzing results. Pall's Technical Support Group is available to assist in conducting trials to select configurations and develop operating conditions necessary to achieve the desired process.

objectives.

#### Cadence Module Materials of Construction

Membrane	Delta regenerated cellulose, 10 kD and 30 kD
Support	Polypropylene
Screens	Polypropylene
Encapsulant	Polyurethane with white pigment (TiO <sub>2</sub> )
Permeate Seals	Medical grade, platinum cured silicone
Gaskets	Medical grade, platinum cured silicone

#### Cadence Module Operating Limits

Maximum Pressure	6 barg (87 psig) @ 23 °C 4.1 barg (60 psig) @ 55 °C
Maximum Transmembrane Pressure (TMP)	4.1 barg (60 psig) @ 4 - 55 °C
Processing Temperature Range	4 to 40 °C (freezing will damage module)
pH Range	2 to 13
Flow Rate Range During Cleaning	≤ 5 L/min/m <sup>2</sup> (0.2 L/min/ft <sup>2</sup> )

The performance of Cadence modules, based on an IgG model feed solution, is demonstrated in the table below over a wide range of feed flow rates (L/min/m<sup>2</sup>) and volumetric concentration factors (X) for various module configurations and feed concentrations (g/L).

Cadence Module Typical Feed Flow Rates in L/min/m <sup>2</sup>	Concentration Factor		
	4X	10X	30X
Process Flux*			
10 LMH	0.22	0.19	0.17
50 LMH	1.11	0.93	0.86
100 LMH	2.22	1.85	1.72

\* LMH = Liters/meter<sup>2</sup>/hour

#### Cadence Module Integrity Test

Systems Integrity Test Pressure	4.1 barg (60 psig)
Acceptable Pressure Decay (System)	< 0.07 barg (1 psig) per minute
Module Integrity Test Pressure	4.1 barg (60 psig)
Acceptable Forward Flow Rate	< 50 sccm/ft <sup>2</sup> (538 sccm/m <sup>2</sup> )

Each Cadence module has a unique serial number for full traceability.

#### Shelf Life

The shelf life of Cadence modules packaged in preservative is expected to be two years from the date of manufacture when the modules are stored unopened in the original packaging at ambient temperature up to 25 °C and protected from direct light. Extended shelf life studies are ongoing.

#### Biological Safety

Materials of construction for Cadence modules have been tested and meet the requirements for the Biological Reactivity Tests listed in the United States Pharmacopeia (USP) under USP <88> for Class VI - 70 °C Plastics.

#### Documentation

Each module is supplied with the following comprehensive documentation to ensure the Cadence module is operated successfully:

- Certificate of Quality
- Care and Use Procedures

- Material Safety Data Sheet for the module preservative

Contact your local Pall representative to obtain:

- Validation Guide
- Validation service for specific tests such as compatibility testing with your product fluid
- Training and technical support to optimize your process using Cadence systems

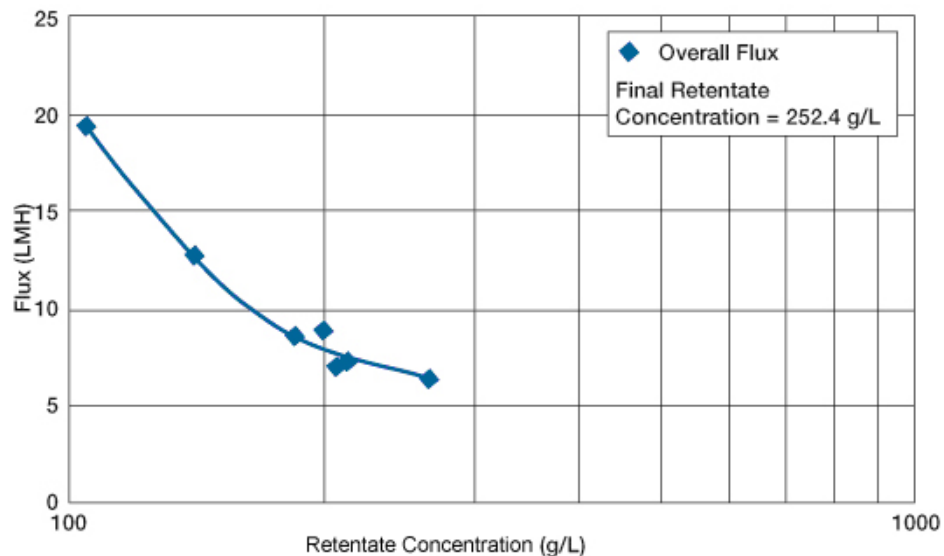
### Pall Provides Comprehensive Documentation with Each Cadence Module



## Performance

### Achieve High Concentration Factors:

**Flux Versus Concentration for Cadence Modules with Delta 30 kD Membrane MAb Material (53.2 g/L Initial Concentration)**



## Application Note

- [Cadence Systems Employ New Single-Pass TFF Technology to Simplify Processes and Lower Costs](#)

## Ordering Information

Identify

and order Cadence modules using the table below. For example, a Cadence T01 module with 10 kD Delta regenerated cellulose membrane area of 0.14 m<sup>2</sup> (1.5 ft<sup>2</sup>) is part number CD010T010815. Note: a Pall Cadence system must be used to operate Cadence modules. (Contact Pall for information on a Cadence system to meet your application requirements.)

### Guide to Cadence Module Part Numbers

Example: Part number CD010T010815

C	D	010	T01	08	15
Concentration	Delta membrane	Nominal molecular weight cutoff (10 kD)	Cassette format	Number of cassettes in series	Total number of cassettes in module

Cadence Module	Configuration	Delta 10 kD Membrane Area (m <sup>2</sup> )	Part Number
T01	4-in-series	0.065	CD 010 T010407
T01	7-in-series	0.12	CD 010 T010713
T01	8-in-series	0.14	CD 010 T010815
T01	9-in-series	0.17	CD 010 T010918
T02	4-in-series	0.13	CD 010 T020407
T02	7-in-series	0.24	CD 010 T020713
T02	8-in-series	0.28	CD 010 T020815
T02	9-in-series	0.33	CD 010 T020918
T12	4-in-series	0.7	CD 010 T120407
T12	7-in-series	1.3	CD 010 T120713
T12	8-in-series	1.5	CD 010 T120815
T12	9-in-series	1.8	CD 010 T120918
T06	4-in-series	3.5	CD 010 T060407
T06	7-in-series	6.5	CD 010 T060713
T06	8-in-series	7.5	CD 010 T060815
T06	9-in-series	9.0	CD 010 T060918
Cadence Module	Configuration	Delta 30 kD Membrane Area (m <sup>2</sup> )	Part Number
T01	4-in-series	0.065	CD 030 T010407
T01	7-in-series	0.12	CD 030 T010713
T01	8-in-series	0.14	CD 030 T010815
T01	9-in-series	0.17	CD 030 T010918
T02	4-in-series	0.13	CD 030 T020407
T02	7-in-series	0.24	CD 030 T020713
T02	8-in-series	0.28	CD 030 T020815
T02	9-in-series	0.33	CD 030 T020918
T12	4-in-series	0.7	CD 030 T120407
T12	7-in-series	1.3	CD 030 T120713
T12	8-in-series	1.5	CD 030 T120815
T12	9-in-series	1.8	CD 030 T120918
T06	4-in-series	3.5	CD 030 T060407
T06	7-in-series	6.5	CD 030 T060713
T06	8-in-series	7.5	CD 030 T060815
T06	9-in-series	9.0	CD 030 T060918

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## **Autoclave Systems – Heaters – Reactors – for Vulcanizing Rubber, Plastic Products and Composites**

WSF has the experience and job-proven track record of manufacturing rubber vulcanizing equipment for production, R&D or process simulation. We can provide a complete system or furnish exactly what you need to maximize your efficiency, versatility and profitability.

- Vulcanizers to ASME Code Standards in a wide range of sizes and design pressures.
- Vertical or horizontal configurations using steam, air-plus-steam, steam-water or “dry” heat.
- Controls to any degree of sophistication.
- Includes patented RAPIDOOR® for quick access and external RAPID/LOADER®.
- Innovations include our unique RAPID/CURE® continuous vulcanizer for wire, cable and hose.

*Automated autoclave system for processing rubber hose features a vertical-slide RAPIDOOR and unique wheel-less, cart-loading mechanism and ergonomic load-positioning system.*





# nProtein A Sepharose 4 Fast Flow

nProtein A Sepharose™ 4 Fast Flow is native protein A coupled to Sepharose 4 Fast Flow. It has nearly twice the total IgG binding capacity of Protein A Sepharose CL-4B, and is the ideal adsorbent for recovery and purification of monoclonal antibodies from cell culture at both laboratory and process scale.

nProtein A Sepharose 4 Fast Flow has been developed and tested in cooperation with leading manufacturers of purified monoclonal antibody products, and is used in routine commercial production.

nProtein A Sepharose 4 Fast Flow replaces Amersham Biosciences' former Protein A Sepharose 4 Fast Flow.

## Features

- Low leakage of protein A
- Used in large scale FDA approved processes
- Manufactured without using any animal-derived components

## The product

Native protein A has a molecular weight of 42 000 daltons, and a structure consisting of several regions (see Fig. 1). Five of these (E, D, A, B and C) show strong specific affinity for the Fc part of IgG, leaving the antigen combining sites within the regions free (1, 2, 3). One molecule of immobilized protein A binds at least two molecules of IgG.

Staphylococcal Protein A

S	E	D	A	B	C	Xr	Xc
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**Fig 1.** Schematic drawing of regions encoded by the gene for *Staphylococcal* protein A. S is the signal sequence. E, D, A, B and C are the homologous repetitive immunoglobulin binding regions. Xr and Xc are C-terminal located, non-immunoglobulin binding regions thought to be responsible for the binding of Protein A to the bacterial cell (4).



Amersham Biosciences' native protein A is produced by fermenting a selected strain of *Staphylococcus aureus*. The purified protein is coupled to Sepharose 4 Fast Flow by the cyanogen bromide technique, giving a highly stable medium with minimal non-specific adsorption. nProtein A Sepharose 4 Fast Flow is manufactured without using any animal-derived components.

The swollen medium has a protein A content of approximately 6 mg/ml drained medium. The total binding capacity for human IgG is approximately 35 mg/ml drained medium.

Sepharose 4 Fast Flow is a highly cross-linked, 4% agarose derivative with impressive kinetics, leading to excellent chromatographic qualities in the immobilized affinity adsorbent. Its rigidity also makes it ideal for process scale applications. nProtein A Sepharose 4 Fast Flow is particularly suitable for recovery and purification of monoclonal antibodies from cell culture supernatants. The rigidity and high degree of substitution of the Sepharose 4 Fast Flow matrix enables the rapid processing of large volumes of dilute cell culture fluid.



## Stability

nProtein A Sepharose 4 Fast Flow has high chemical and mechanical stability. It withstands high concentrations of hydrogen bond disrupting agents such as urea, guanidine hydrochloride and sodium thiocyanate. It has high thermal stability, but is not autoclavable. The characteristics of the product are summarized in Table 1.

**Table 1.** Characteristics of nProtein A Sepharose 4 Fast Flow

Ligand	native Staphylococcal protein A
Degree of substitution	approx. 6 mg native protein A/ml drained medium
Ligand coupling method	cyanogen bromide activation
Dynamic binding capacity*	min 20 mg human IgG/ml drained medium
Matrix	highly cross-linked 4% agarose
Average particle size	90 µm (45–165 µm)
Chemical stability	no significant change in chromatographic performance after 1 week storage using 8 M urea, 6 M guanidine-HCl, 2% benzyl alcohol or 20% ethanol
pH stability**	
Working	2–9
Long term	3–9
Short term	2–10
Max linear flow rate	1300 cm/h at 25 °C, XK 16/20 column, bed height 5 cm
Max operating back pressure	0.1 MPa (1 bar, 14 psi)
Sanitization	wash the packed column with 2% hibitane/20% ethanol or 70% ethanol
Storage	20% ethanol at +4 to +8 °C

\* The binding capacity was determined at a linear flow rate of 100 cm/h, column: 7.5 x 50 mm, sample volume: 250 µl, sample concentration: 1 mg human IgG/ml

\*\* Complete data on the stability of protein A as a function of pH are not available. The ranges given are estimates based on our knowledge and experience. Please note the following:

pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures

pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyse at very low pH

## Process scale use

### a) Columns

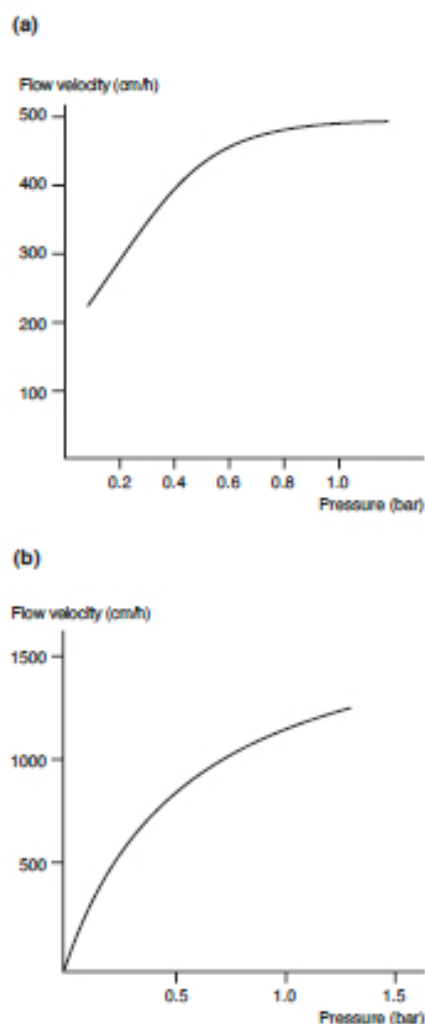
Columns recommended for nProtein A Sepharose 4 Fast Flow are shown in Table 2.

**Table 2.** Recommended Amersham Biosciences columns for nProtein A Sepharose 4 Fast Flow.

Column	Bed height	Medium volume
XK Column 50/30 Fast Flow*	5–15 cm	100–300 ml
BPG™ 100/500		Up to 2.4 l

\* These are columns fitted with a special Fast Flow adaptor to increase throughput.

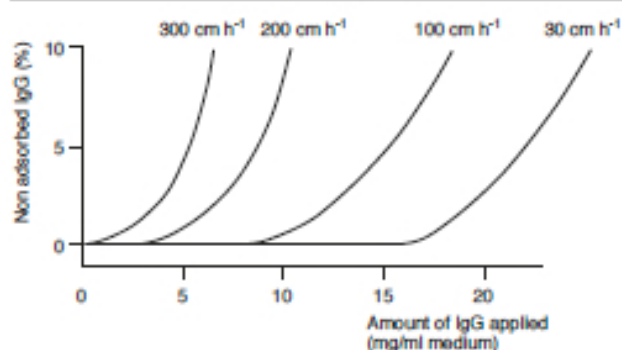
When packing the medium at a 5 cm bed height, the recommended packing flow velocity is at least 700 cm/h, and at a 15 cm bed height at least 300 cm/h. The working flow velocity should not exceed 80% of the packing flow velocity. As a guide pressure/flow velocity curves for the Sepharose 4 Fast Flow base matrix packed in XK 50/30 Fast Flow and BP 113 columns are shown in Figure 2.



**Fig 2.** Pressure/flow velocity curve for Sepharose 4 Fast Flow in XK 50/30, bed height 15 cm (a) and BP 113, bed height 5 cm (b); mobile phase H<sub>2</sub>O. (Work from Amersham Biosciences AB).

### b) Dynamic capacity

The dynamic capacity of chromatographic adsorbents is a function of the flow velocity used for loading samples, and increases with decreasing flow velocity. Furthermore, individual antibodies differ in their affinity to protein A. To obtain an optimal purification scheme with respect to capacity and time, it is necessary to first determine the capacity for the specific antibody to be purified over a range of different flow velocities (see the example in Fig. 3). Once this is known it is then possible to control the flow velocity during the loading phase to achieve maximum binding of the antibody in minimum time. In practice, this means initially loading the sample at a high flow velocity (e.g. 300 cm/h) and reducing the flow velocity successively with increasing sample load.



**Fig 3.** One example of how the capacity for human IgG depends on the flow velocity with nProtein A Sepharose 4 Fast Flow. The non-adsorbed IgG (%) was measured as a function of the amount applied to the column at 4 different flow velocities, 300, 200, 100 and 30 cm/h. Concentration of the applied sample: 0.33 mg IgG/ml. Column: HR 5/5. Buffer system: 0.1 M  $\text{Na}_2\text{HPO}_4$ , pH 7.0. (Work from Amersham Biosciences AB).

### c) Operation

nProtein A Sepharose 4 Fast Flow is supplied in suspension in 20% ethanol.

1. After packing, wash the medium bed with at least three column volumes of starting buffer to remove preservative.
2. Note the following points when loading the sample:
  - The sample pH should be the same as the starting buffer pH.
  - The sample should be filtered through a 0.22 – 0.45  $\mu\text{m}$  filter. (This prolongs the working life of the medium).
3. After loading the sample, wash the medium with starting buffer until the base line is stable.
4. When eluting the sample, reverse the direction of flow.

### d) Process Hygiene

Good process hygiene ensures the safety and integrity of the final product by removing or controlling any unwanted substances which might be present or generated in the raw material, or derived from the purification system itself. In practice, process hygiene of most affinity media usually means reduction of product contamination by sanitization, followed by a cleaning step.

#### Sanitization

Sanitization is the reduction of microbial populations on the medium. Two suggested alternative protocols are:

- i) Equilibrate with a buffer consisting of 2% hibitane digluconate and 20% ethanol.
  - ii) Allow to stand for 6 hours.
  - iii) Wash with sterile buffer.
- or
- i) Equilibrate with 70% ethanol.
  - ii) Allow to stand for 12 hours.
  - iii) Wash with sterile buffer.

#### Cleaning

The general recommendation for cleaning nProtein A Sepharose 4 Fast Flow is to use a mixture of 50 mM NaOH and 1 M NaCl. As an alternative cleaning protocol 6 M guanidine hydrochloride can be used. Phosphoric acid (100 mM) has also been used for cleaning. To remove hydrophobically bound substances a solution of non-ionic detergent or ethanol is recommended.

### e) Regeneration

After each separation cycle, regenerate the medium bed by washing with approximately 3 column volumes of 0.1 M citrate buffer, pH 3 until the base line is stable.

### f) Storage

For longer periods of storage, keep nProtein A Sepharose 4 Fast Flow in a suitable bacteriostat, e.g. 20% ethanol, at 4–8 °C. The medium must not be frozen.

## Applications

The most important application area for nProtein A Sepharose 4 Fast Flow is the purification of monoclonal antibodies from cell culture (5, 6). High IgG capacity and high flow velocities make the medium ideal for both laboratory and process scale separations.

There is a natural diversity between the different subclasses of IgG and even within subclasses. Therefore the binding and elution system must be optimized for every monoclonal antibody to be purified.

## Data Sheet

# Eshmuno® S resin

## For superior downstream mAB purification

Eshmuno® is a unique family of ion-exchange resins specifically designed for highly productive downstream bioprocessing. The cation exchanger Eshmuno® S is the first member of the Eshmuno® resin family and is highly productive in direct capture and post-protein A steps.

### Benefits

- Superior productivity for mAB downstream processing
- More selectivity and HCP removal
- Active tentacle adsorption
- Robust and safe packing procedures
- Tangible savings in cost and development time



### Eshmuno® S resin characteristics

Type	Strong cation exchanger
Functional group	-SO <sub>3</sub>
Base matrix	Surface grafted rigid polyvinyl ether hydrophilic polymer
Lysozym capacity	115–165 mg/mL settled resin
Ionic capacity	50–100 µeq/mL settled resin
Mean particle size	75–95 µm
IgG dynamic capacity	>60 mg/mL (2 min. residence time)
Pressure drop (100 x 16 mm, 5 mL/min., 150 cm/h)	<1.0 bar

## Superior productivity for mAB downstream processing

Eshmuno® S resin exhibits a superior binding capacity for antibodies compared to other modern cation-exchangers. Fig. 1 shows the dynamic binding capacity (DBC) for direct capture of a monoclonal antibody mAB02 at 5% breakthrough and 5 min. residence time from a real diluted feedstock. The DBC of Eshmuno® S resin is approximately 50% higher than the capacity of other surface-grafted cation exchangers.

A similar superior binding capacity can be shown in post-protein A purification steps. Fig. 2 illustrates the increased binding capacity of Eshmuno® S resin in an intermediate purification step of mAB03.

## Pressure versus flow curve of Eshmuno® S resin

In combination with the excellent pressure flow behaviour (Fig. 3) an outstanding productivity of more than 40 mg/mL x h (dimension for productivity) for Eshmuno® S resin can be achieved, resulting in considerable manufacturing cost savings in mAB production.

### Superior mAB binding capacity in direct capture step

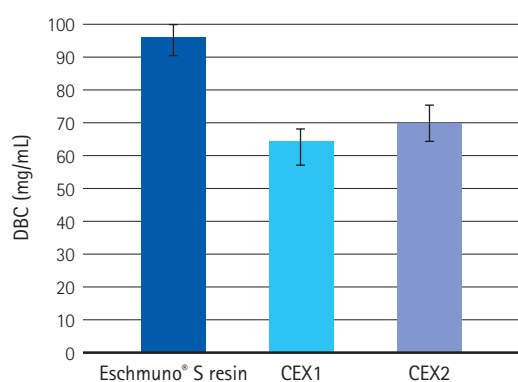


Figure 1.

mAB02 DC, 5% breakthrough, 4.3 mS/cm, pH 6.0 [mAB02] = 0.62 mg/mL, 5 min. residence time, 1 mL scout column

### Binding capacity of purified mAB03 on Eshmuno® S resin

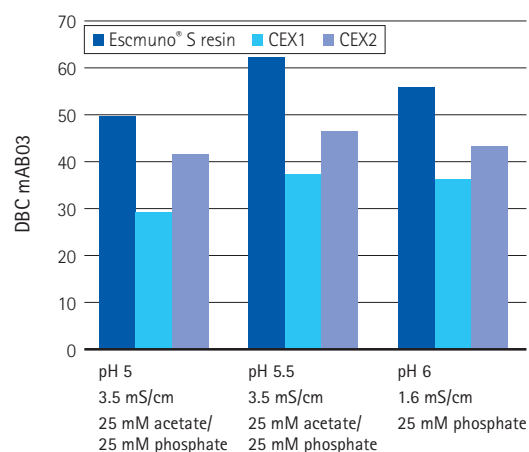


Figure 2.

DBC of mAB03 5 mg/mL in buffer A, 2 min. residence time, 1 mL scout column

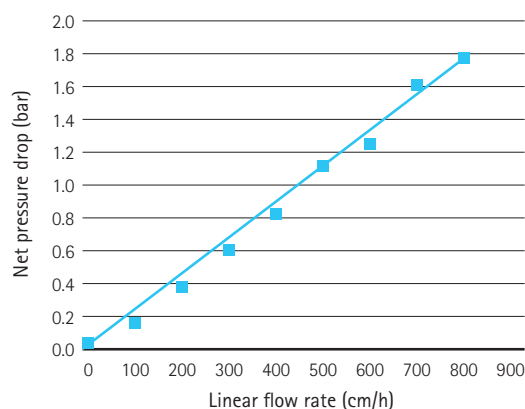


Figure 3.

20 cm i.d. column; 19,5 cm bed height; 8% compression recorded in 150 mM NaCl

## More selectivity and HCP removal

A crucial property of any ion exchange material in biochromatography is the ability to specifically select the biomolecule of interest. While Eshmuno® S resin carries the same functional group like Fractogel® SO<sub>3</sub> resin, a slightly modified selectivity can be observed (Fig. 4), which allows a wider flexibility for the specific purification challenge.

The result: Eshmuno® S resin is the most efficient resin in the removal of host cell proteins (Fig. 5).

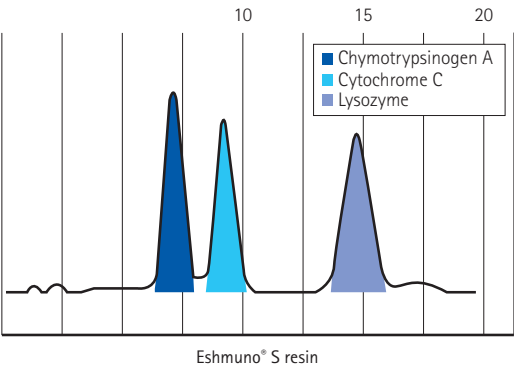


Figure 4.

A mixture of ■ Chymotrypsinogen A, ■ Cytochrome C, and ■ Lysozyme was separated under standard conditions

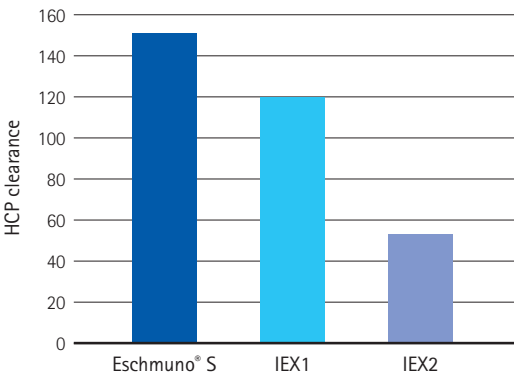


Figure 5.

HCP Clearance factor of mAB02, 5% breakthrough, 4.3 mS/cm, pH 6.0, 5 min. residence time, 1 mL scout column

## Active tentacle technology

Merck KGaA was the first manufacturer of a biochromatography resin (Fractogel®) with tentacle structure (Fig. 6). The main advantage of this tentacle chemistry is the increased amount of sterically accessible ligands to more effectively bind the biomolecule of interest thus increasing the capacity of the resin.

Eshmuno® S resin combines both, the reliable tentacle technology with the properties of a new hydrophilic polyvinyl ether base matrix. The polymer matrix allows the use of much higher flow rates, while the biomolecule is still strongly bound by the tentacle.



Figure 6.

Resin tentacles forming a three-dimensional ion exchange network, enable easy access of the proteins to the ligands

## Robust and safe packing procedures

Eshmuno® S resin can be easily packed into production scale columns for biochromatography either by simple flow packing or axial compression. To prevent corrosion of the tubing system, Eshmuno® columns can be packed using 0.01 M sodium hydroxide solutions and even pure water resulting in plate numbers >2400/m with good peak symmetry.

For the packing of Eshmuno® and sanitization of the column we recommend EMD Millipore chemicals especially dedicated for the use in biopharmaceutical production with the brandname EMPROVE® bio.

## Tangible savings in cost and development time

With the use of Eshmuno® S resin in downstream processing considerable manufacturing cost savings can be achieved. The productivity of purification in a model process of a monoclonal antibody could be increased 5-fold by using Eshmuno® S resin instead of a conventional soft-gel ion exchanger. The use of Eshmuno® S resin instead of a protein A based capture step can save up to 30% of your purification costs.

## Ordering Information

Description	Catalogue No.
Eshmuno® S resin	1.20078



[www.millipore.com](http://www.millipore.com)

## To Place an Order or Receive Technical Assistance

In the U.S. and Canada, call toll-free **1-800-645-5476**

For other countries across Europe and the world, please visit [www.millipore.com/offices](http://www.millipore.com/offices)

For Technical Service, please visit [www.millipore.com/techservice](http://www.millipore.com/techservice)



# Capto™ S Capto Q Capto DEAE

Capto S, Capto Q and Capto DEAE are, respectively, strong cation, strong anion and weak anion exchange BioProcess™ media for capture and intermediate purification of proteins from large feed volumes by packed bed chromatography.

All Capto media provide:

- Raised productivity with high dynamic binding capacity at high flow.
- Increased yield with rapid mass transfer.
- Reduced process time with high volume throughput.
- Cost-effective processing with smaller unit operations.
- For effective and rigorous CIP procedures.



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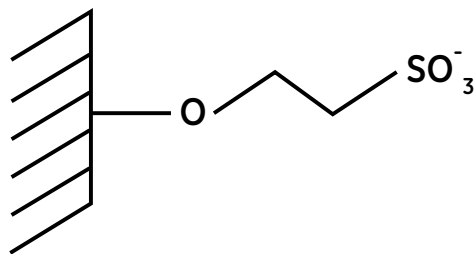


# 1 BioProcess media

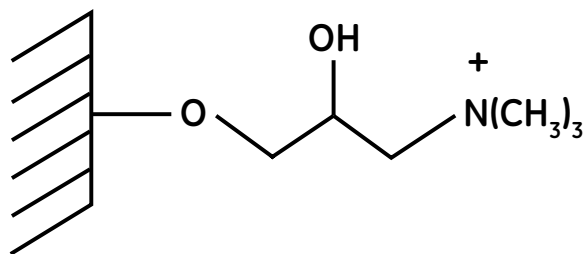
BioProcess media are developed and supported for production-scale chromatography. All BioProcess media are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of media for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess media cover all purification steps from capture to polishing.

## 2 Properties of Capto S, Capto Q and Capto DEAE

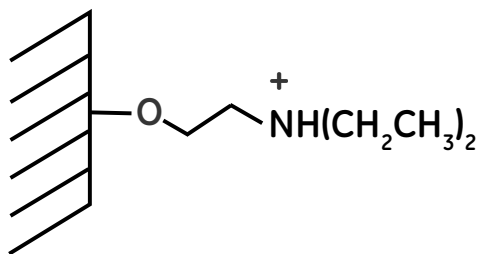
For ion exchange chromatography, Capto S uses a sulfonate group (Fig 1a), Capto Q uses a quaternary amine group (Fig 1b) and Capto DEAE uses a diethylaminoethyl group (Fig 1c).



**Fig 1a.** The strong cation exchange group of Capto S.



**Fig 1b.** The strong anion exchange group of Capto Q.

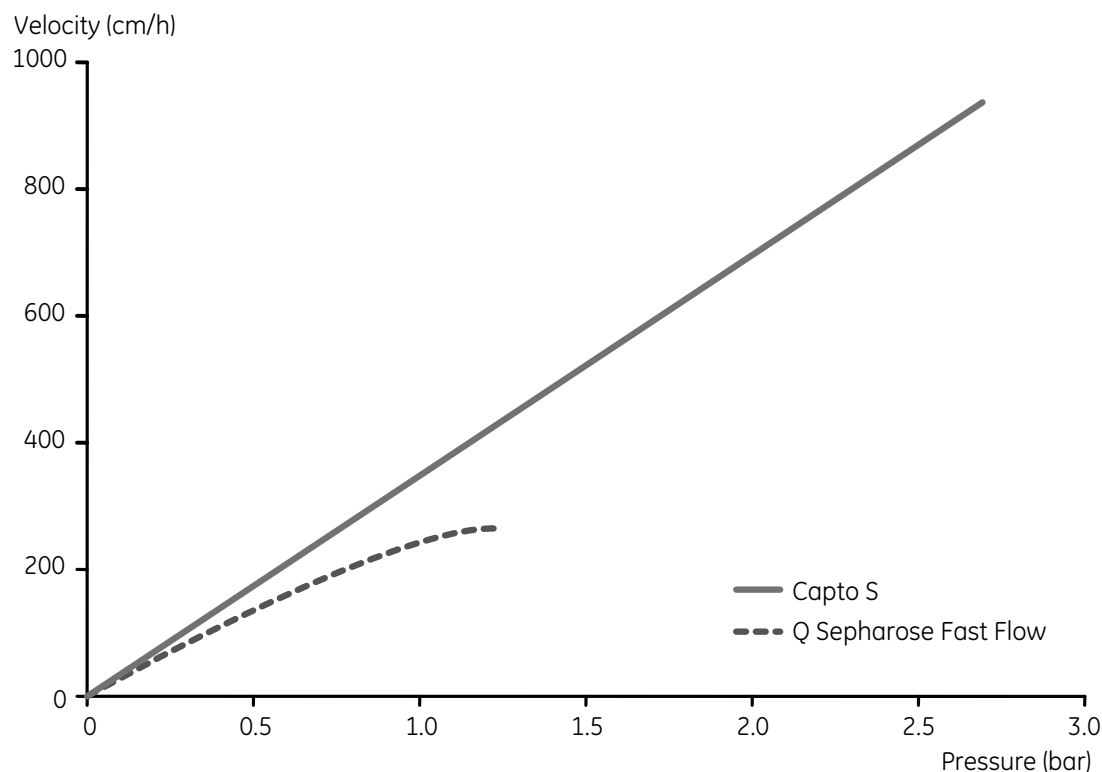


**Fig 1c.** The weak anion exchange group of Capto DEAE.

Capto S, Capto Q and Capto DEAE are designed to increase speed, capacity, and throughput in capture and intermediate purification of biomolecules. By offering high capacity at high flow velocities with low back pressure, process cycle times may be reduced and productivity increased.

Capto S, Capto Q and Capto DEAE are based on the same high flow agarose matrix which is designed to give low back pressures at high fluid velocities (700 cm/h in a 1 m diameter column with 20 cm bed height at < 3 bar in water). Note that the back pressure will vary with column type and medium bed height. Figure 2 shows the pressure/flow curve for Capto S packed in AxiChrom™ 1000.

All media have dextran surface extenders for increased capacity, and fast mass transfer.



**Fig 2.** Pressure/flow curve for Capto S compared to Q Sepharose™ Fast Flow. *Running conditions:* AxiChrom 1000 for Capto S, Chromaflow™ 1000 for Q Sepharose Fast Flow, 20 cm packed bed, with water at 20°C. The pressure includes pressure drop from the bed and the column. System/tubing pressure is excluded.

The highly cross-linked agarose base matrix gives the media high chemical and physical stability. Characteristics such as capacity, elution behavior, and pressure/flow rate are unaffected by the solutions commonly used in process chromatography and cleaning procedures (Tables 1, 2 and 3).

**Table 2.** Characteristics of Capto Q

Matrix	High flow agarose with a dextran surface extender
Ion exchange type	Strong anion, Q
Charged group	$-N^+(CH_3)_3$
Total ionic capacity	0.16 to 0.22 mmol $Cl^-$ /ml medium
Particle size <sup>1</sup>	90 $\mu m$ ( $d_{50v}$ )
Flow velocity	700 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa)
Dynamic binding capacity <sup>2</sup>	> 100 mg BSA/ml medium
pH stability <sup>3</sup>	
Working range	2 to 12
Cleaning-in-place	2 to 14
Working temperature	4°C to 30°C
Chemical stability	All commonly used aqueous buffers, 1 M acetic acid, 1 M NaOH <sup>4</sup> , 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol and 70% ethanol
Avoid	Oxidizing agents, anionic detergents
Storage	20% ethanol

<sup>1</sup>  $d_{50v}$  is the median particle size of the cumulative volume distribution.

<sup>2</sup> Dynamic binding capacity at 10% breakthrough as measured at a residence time of 1 minute, 600 cm/h in a Tricorn 5/100 column with 10 cm bed height, in a 50 mM Tris-HCl buffer, pH 8.0.

<sup>3</sup> Working range pH: pH interval where the medium can be operated without significant change in function.

Cleaning-in-place pH: pH stability where the medium can be subjected to cleaning- or sanitization-in-place without significant change in function.

<sup>4</sup> No significant change in dynamic binding capacity and carbon content after 1 week of storage in 1 M NaOH at 40°C.

### 3 Method optimization

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product yield and purity. Design the method in laboratory scale.

For certain proteins, dynamic binding capacities increase at increased conductivity. Therefore, we recommend scouting for optimal dynamic binding conditions on Capto S, Capto Q and Capto DEAE, for the target protein at conductivities between 2 mS/cm and 15 mS/cm as well as scouting for optimal binding pH.

For Capto S and Capto DEAE, the dynamic binding capacities decrease for some proteins at lower temperatures. Screening for buffer concentration at the temperature where the process is intended to be run will give the optimal dynamic binding capacity.

Since Capto S, Capto Q and Capto DEAE allow efficient capture at high fluid velocities, pay special attention to optimizing elution conditions to avoid tailing peaks when eluting the protein of interest.

Balancing product recovery against throughput is the major consideration when optimizing a method. The dynamic binding capacity for the target protein should be determined using process feedstock. Since the dynamic binding capacity is a function of the fluid velocity applied during sample application, the breakthrough capacity must be defined over a range of different residence times to show the optimal level of throughput.

When designing a process it is also important to take the cleaning of the media into consideration. Capto DEAE can be more challenging to clean than Capto S and Capto Q, for more details, see Chapter *Maintenance*.

For more information about method development and optimization, consult the handbook, *Ion exchange Chromatography & Chromatofocusing: Principles and Methods*, (11-0004-21).

## 4 Scale-up

### Principles for scale-up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up is typically performed by keeping bed height and linear fluid velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the linear fluid velocity (cm/h) applied during sample loading.

### Suggested procedure for scale-up

- 1 Select the bed volume according to required binding capacity. Keep sample concentration and gradient slope constant.
- 2 Select a column diameter to obtain a bed height of 10 cm to 40 cm. The high rigidity of the Capto S, Capto Q and Capto DEAE base matrix allows for bed heights well above 20 cm.
- 3 The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery system and monitoring system for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems.

## **Calculating amount of medium**

The amount of medium needed can be calculated by: column cross-sectional area (cm<sup>2</sup>) × bed height (cm) × compression factor (settled medium bed height/ packed medium bed height).

## **Washing the medium**

Equilibrate all materials to room temperature. Mount the glass filter funnel onto the filtering flask. Pour the medium into the funnel and wash with approximately 5 to 10 ml 10 mM NaCl per ml medium.

## **Preparing the packing slurry**

The slurry concentration should be 40 to 60% in 10 mM NaCl, measured in a measuring cylinder after settling overnight.

## **Equipment needed**

An ÄKTA design system or a stand-alone pump that can deliver 20 ml/min is used. The pump filter unit and the flow restrictor should be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure.

### **For packing Tricorn 5/100 column**

Tricorn 5/100 column, Tricorn 5/100 glass tube (used as a packing reservoir), packing connector 5-5 and bottom unit.

### **For packing Tricorn 10/100 column**

Tricorn 10/100 column, Tricorn 10/100 packing equipment, which includes the 10 mm packing connector, Tricorn 10/100 glass tube (used as a packing reservoir), and bottom unit with filter holder, cap and stop plug.

When working with large volumes, real feed or repeated loading, Tricorn coarse filter kits are recommended to reduce the risk of clogging. Use Tricorn 5 Coarse Filter Kit (11-0011-53) or Tricorn 10 Coarse Filter Kit (11-0012-54).

## Packing procedure

To pack the column, use 10 mM NaCl in distilled water and proceed as follows:

- 1 Rinse the column and packing tube in 10 mM NaCl.
- 2 Insert a bottom filter into the filter holder and wet the filter.
- 3 Wet the O-ring on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
- 4 Insert the filter holder into the column tube. Ensure that the “keyed” part of the filter holder fits into the slot on the threaded section on the column tube. Screw the end cap onto the column tube.
- 5 Pour the packing solution into the tube and ensure that the liquid drips from the column. Insert a stop plug into the bottom unit when approximately 1 cm of packing solution remains.
- 6 Screw a suitable Tricorn packing connector onto the top of the column tube. The Tricorn packing connector must be fitted with suitable O-rings (included with the Tricorn packing connector). Screw the Tricorn packing tube into the upper fitting of the Tricorn packing connector.
- 7 Mount the column and packing unit vertically on a lab stand.
- 8 Fill both column tube and packing tube with slurry. Avoid formation of air bubbles in the gel by pouring it along a thin capillary.
- 9 Attach an extra bottom unit or an adapter unit to the top of the packing tube. Fill the capillary from the pump with packing solution and connect the pump to the top of the packing unit, remove the stop plug from the bottom of the column tube.
- 10 Pack the medium at 540 cm/h (1.8 ml/min in Tricorn 5/100, 7.1 ml/min in Tricorn 10/100). When the liquid above the medium bed is clear, continue packing for 10 min.
- 11 Pack the medium for an additional 10 min at 3000 cm/h (Tricorn 5/100: 9.8 ml/min, Tricorn 10/100: 39.3 ml/min).



- 12 Switch off the pump and connect a stop plug into the bottom unit. Remove the packing tube and packing connector. If necessary, remove excess medium with a Pasteur pipette or spatula by re-suspending the top of the packed bed. Ensure that the medium surface is as even as possible.
- 13 Add packing solution to the upper edge of the column tube.
- 14 Place a pre-wetted filter on top of the fluid in the column.  
**Note:** *The top coarse filter is inserted by another procedure. See separate instruction included in Tricorn coarse filter kits.*
- 15 Prepare the adapter unit by screwing the guiding ring, inside the adapter unit, down to the lower end position.
- 16 Wet the O-ring on the adapter unit by dipping it in water, packing solution, or 20% ethanol.
- 17 Screw the guiding ring back 1.5 turns.
- 18 Mount the top adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. Ensure that there are no air bubbles.  
**Note:** *The top adapter should be connected but not fully screwed down.*
- 19 Connect the pump, remove the stop plug and start a flow (Tricorn 5/100: 1 ml/min (300 cm/h), Tricorn 10/100: 5 ml/min (380 cm/h)).
- 20 Slowly screw the adapter unit down until the filter meets the bed surface. Ensure that the filter meets the bed horizontally.
- 21 Increase the flow to 3000 cm/h (Tricorn 5/100: 9.8 ml/min, Tricorn 10/100: 39.3 ml/min).
- 22 If the medium bed compresses, slowly screw the adapter unit down to the medium surface with maintained flow.
- 23 Pack the medium for 5 min. If the bed has compressed further, screw the adapter unit down to the medium surface.
- 24 Stop the flow and connect a stop plug to the bottom unit.

## 7 Maintenance

For best performance from Capto S, Capto Q and Capto DEAE, and to maximize the working life time of the media, follow the procedures described below.

### Equilibration

After packing, and before a chromatographic run, equilibrate with equilibration buffer by washing with at least five bed volumes for Capto S and Capto Q and at least 10 bed volumes for Capto DEAE, or until the column effluent shows stable conductivity and pH values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with equilibration buffer until the conductivity and pH values are stable.

### Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 1–2 M NaCl in buffer). Regenerate the medium by washing with at least five bed volumes of equilibration buffer for Capto S, Capto Q and Capto DEAE, or until the column effluent shows stable conductivity and pH values.

### Cleaning-In-Place

Cleaning-In-Place (CIP) is a procedure that removes contaminants such as lipids, endotoxins, and precipitated or denatured proteins that remain in the packed column after regeneration. This type of contamination occurs frequently when working with feedstock. Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain the capacity, flow properties, and general performance of Capto S, Capto Q and Capto DEAE.

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends on the nature and the condition of the feedstock, but for capture steps, CIP is recommended after each cycle.

**Note:** *For some contaminants a more rigorous CIP procedure can be required for Capto DEAE than for Capto S and Capto Q, see CIP protocols, below.*

## CIP protocols

Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1 M NaOH at 40 cm/h with reversed flow direction. Contact time 1 to 4 h, dependent on feed. If the removal of contaminants is not satisfactory for Capto DEAE, use 1 M NaOH containing 1 M NaCl as CIP solution.
Ionically bound proteins	Wash with 0.5 to 2 column volumes (CV) of 2 M NaCl with reversed flow direction. Contact time 10 to 15 min.
Lipids and very hydrophobic proteins	Wash with 2 to 4 CV of up to 70% ethanol <sup>1</sup> or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h, dependent on feed. Alternatively, wash with 2 to 4 CV of 0.1% nonionic detergent with reversed flow direction. Contact time 1 to 2 h, dependent on feed.

<sup>1</sup> Specific regulations may apply when using 70% ethanol since the use of explosion-proof areas and equipment may be required.

## Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1 M NaOH with a contact time of 1 h is recommended. The CIP protocol for precipitated, hydrophobically bound proteins or lipoproteins sanitizes the medium effectively.

## Storage

Store unused medium in the container at a temperature of 4°C to 30°C. Ensure that the cap is fully tightened. Packed media and bulk media should be stored in 20% ethanol containing 0.2 M sodium acetate (Capto S) or 20% ethanol (Capto Q and Capto DEAE). After storage, equilibrate with at least five column volumes of starting buffer for Capto S and Capto Q and at least 10 bed volumes of starting buffer for Capto DEAE before use.

## 8 Ordering information

Product	Quantity	Code No
Capto S	25 ml	17-5441-10
	100 ml	17-5441-01
	1 l	17-5441-03
	5 l	17-5441-04
	10 l	17-5441-05
	60 l	17-5441-60
Capto Q	25 ml	17-5316-10
	100 ml	17-5316-02
	1 l	17-5316-03
	5 l	17-5316-04
	10 l	17-5316-05
	60 l	17-5316-60
Capto DEAE	25 ml	17-5443-10
	100 ml	17-5443-01
	1 l	17-5443-03
	5 l	17-5443-04
	10 l	17-5443-05
	60 l	17-5443-60

Capto S bulk media products are supplied in suspension in 20% ethanol containing 0.2 M sodium acetate. Capto Q and Capto DEAE bulk media products are supplied in suspension in 20% ethanol. For additional information, including a Data File, please contact your local GE Healthcare representative.

Related product	Quantity	Code No
HiScreen Capto S	1 x 4.7 ml	28-9269-79
HiScreen Capto DEAE	1 x 4.7 ml	28-9269-82
Tricorn 5/100 column	1	18-1163-10
Tricorn 10/100 column	1	18-1163-15
HiScale 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

Related literature	Code No
Movie	Column Packing – The Movie 18-1165-33
Handbook	Ion Exchange Chromatography & Chromatofocusing: Principles and Methods 11-0004-21
Data file	Capto Q, Capto S and Capto DEAE 11-0025-76
Application notes	High productivity capture of $\alpha$ -chymotrypsin on Capto S cation exchange 28-4078-15
	Screening and optimization of the loading conditions on Capto S 28-4078-16
	Capto S cation exchanger for post-Protein A purification of monoclonal antibodies 28-4078-17
	High-productivity capture of Green Fluorescent Protein on Capto Q 11-0026-20
	Screening of loading conditions on Capto S using a new high-throughput format, PreDictor plates 28-9258-40
	Process-scale purification of monoclonal antibodies - polishing using Capto Q 28-9037-16
	Purification of a monoclonal antibody using ReadyToProcess columns 28-9198-56
	Use of Capto ViralQ for the removal of genomic DNA from influenza virus produced in MDCK cells 28-9769-69

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28-4074-52 AD 02/2012

## Ab Buffer Kit

### Introduction

Ab Buffer Kit contains 10X stock solutions of Binding and Elution buffers and ready to use Neutralizing buffer. The buffers are optimized for rapid purification of monoclonal and polyclonal IgG using immobilized Protein A or Protein G.

The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible and convenient purification work. The buffers have been prepared using the highest quality chemicals and water, and have been filtered through a 0.45 µm filter.

The following protocols can be used with buffers from Ab Buffer Kit:

- Protocol 1, using 1 ml or 5 ml HiTrap™ columns.
- Protocol 2, using SpinTrap™ columns (Protein A HP SpinTrap, Protein G HP SpinTrap and Ab SpinTrap).
- For Ab purification using multiwell plates, please follow the instruction provided with the Protein A HP or Protein G HP MultiTrap™ product.

### Kit content

Buffer	Buffer content	Formulation	Volume
Binding Buffer	0.2 M sodium phosphate, pH 7.0*	10X	50 ml
Elution Buffer	1 M glycine-HCl, pH 2.7	10X	15 ml
Neutralizing buffer	1 M Tris-HCl, pH 9*	Ready to use	25 ml

\* Containing 20% ethanol as a preservative

### Protocol 1

Use this protocol for:

- HiTrap Protein G HP
- HiTrap Protein A HP
- HiTrap Protein A FF

### Recommendations

- Recommended flow rates:
  - Approx. 1–3 ml/min for HiTrap 1 ml columns
  - Approx. 5–10 ml/min for HiTrap 5 ml columns.
- Prepare buffers according to Table 1 or 2.
- Prepare collection tubes by adding 60 to 200 µl of Neutralizing buffer per ml of fraction to be collected. In this way the final pH of the eluted antibodies will be approximately neutral, which preserves the activity of acid labile IgG.
- Check also the relevant column instructions.

### Buffer preparation.

**Table 1.** Buffer preparation for 1 purification on 1 ml HiTrap columns.

Buffer	Stock solution	Distilled water	Final volume
Binding buffer	2.5 ml	22.5 ml	25 ml
Elution buffer	0.5 ml	4.5 ml	5 ml

**Table 2.** Buffer preparation for 1 purification on 5 ml HiTrap columns.

Buffer	Stock solution	Distilled water	Final volume
Binding buffer	12.5 ml	112.5 ml	125 ml
Elution buffer	2.5 ml	22.5 ml	25 ml

### Protocol

- 1 Prepare the sample by centrifugation (10 000 × g, 20 min) or filter (0.45 µm) if there are particles present or the appearance is cloudy.
  - If the sample is serum or ascites fluid, dilute the sample 1:1 with Binding buffer.
- 2 Fill the syringe with Binding buffer. Remove the stopper and connect the column to the syringe (with the provided adapter) "drop to drop" to avoid introducing air into the column.
- 3 Remove the snap-off end at the column outlet.
- 4 Wash the column with 10 column volumes (CV) of Binding buffer (1 CV = 1 ml for HiTrap 1-ml column; 1 CV = 5 ml for HiTrap 5-ml column).
- 5 Apply the sample, using a syringe fitted to the Luer adapter. Collect the flowthrough.
- 6 Wash with 5 to 10 CV of Binding buffer or until no material appears in the effluent. Collect wash fractions.
- 7 Elute with 2 to 5 CV of Elution buffer. Collect 1-ml fractions in collection tubes containing Neutralizing buffer.



## Protocol 2

Use this protocol for:

- Ab SpinTrap
- Protein A HP SpinTrap
- Protein G HP SpinTrap

### Recommendations

- The protocol is optimized for room temperature. If performed at a lower temperature, a longer incubation time may be needed.
- Use Ab SpinTrap columns with a standard microcentrifuge. Place a column in a 2-ml microcentrifuge tube to collect liquid during centrifugation.
- Prepare buffers according to Table 3.
- Prepare two collection tubes per sample for eluted fractions by adding 30  $\mu$ l Neutralizing buffer to a 2-ml microcentrifuge tube.
- Check also the relevant column instructions.

### Buffer preparation

**Table 3.** Buffer preparation for 10 purifications on Ab SpinTrap columns or 20 reactions using MultiTrap multiwell plates.

Buffer	Stock solution	Distilled water	Final volume
Binding buffer	2 ml	18 ml	20 ml
Elution buffer	1 ml	9 ml	10 ml

## Protocol

- 1 Invert and shake the Ab SpinTrap column repeatedly to resuspend the medium.
- 2 Loosen the top cap one-quarter of a turn and break off the bottom closure.
- 3 Place the column in a 2-ml microcentrifuge tube and centrifuge for 30 s at 70 to 100  $\times$  g to remove the storage liquid.
- 4 Remove the top cap. Equilibrate the column by adding 600  $\mu$ l Binding buffer. Centrifuge for 30 s at 70 to 100  $\times$  g.
- 5 Add the sample. Maximum sample volume is 600  $\mu$ l/load.
- 6 Secure the top cap tightly and incubate for 4 min with gentle mixing (mixing with an end-over-end mixer machine is recommended but other methods can also be used).
- 7 Loosen the top cap one-quarter of a turn, centrifuge for 30 s at 70 to 100  $\times$  g.  
Several sample applications can be performed provided that the capacity of the column is not exceeded. Repeat this step after each application. Empty the microcentrifuge tube as needed.
- 8 Wash with 600  $\mu$ l Binding buffer. Centrifuge for 30 s at 70 to 100  $\times$  g.
- 9 Repeat the wash.
- 10 Elute the bound antibody by adding 400  $\mu$ l Elution buffer to the column.
- 11 Place the column in a 2-ml microcentrifuge tube containing 30  $\mu$ l Neutralizing buffer.
- 12 Centrifuge for 30 s at 70  $\times$  g.  
The eluted fraction contains the purified antibody.
- 13 Repeat this step one time, collecting the second elution in a fresh 2-ml microcentrifuge tube containing 30  $\mu$ l Neutralizing buffer.

Most of the bound antibody is eluted after two elution steps.



## Ordering information

### Products

Description	Quantity	Code No.
Ab Buffer Kit	1	28-9030-59

### Related products

Description	Quantity	Code No.
Protein A HP SpinTrap	16 columns	28-9031-32
Protein G HP SpinTrap	16 columns	28-9031-34
Ab SpinTrap	50 columns	28-4083-97
Protein A HP MultiTrap	4 × 96-well plates	28-9091-33
Protein G HP MultiTrap	4 × 96-well plates	28-9091-35
HiTrap Protein G HP, 1 ml	2 columns	17-0404-03
HiTrap Protein G HP, 1 ml	5 columns	17-0404-01
HiTrap Protein G HP, 5 ml	1 column	17-0405-01
HiTrap Protein G HP, 5 ml	5 columns	17-0405-03
HiTrap Protein A HP, 1 ml	2 columns	17-0402-03
HiTrap Protein A HP, 1 ml	5 columns	17-0402-01
HiTrap Protein A HP, 5 ml	1 column	17-0403-01
HiTrap Protein A HP, 5 ml	5 columns	17-0403-03
HiTrap rProtein A FF, 1 ml	2 columns	17-5079-02
HiTrap rProtein A FF, 1 ml	5 columns	17-5079-01
HiTrap rProtein A FF, 5 ml	1 column	17-5080-01
HiTrap rProtein A FF, 5 ml	5 columns	17-5080-02

### Literature

Title	Code No.
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86

[www.gelifesciences.com/sampleprep](http://www.gelifesciences.com/sampleprep)

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imagination at work

# PLANOVA™ Filters

**VIRUS REMOVAL FOR  
BIOTHERAPEUTIC  
PRODUCTS**



# Planova™ BioEX Filters

## ■ PVDF Hollow Fiber Filter

### Construction

The Planova™ BioEX filter membrane is made of hydrophilized polyvinylidene fluoride (PVDF), a robust material with a unique structure that permits high-flow-rate filtration of high-concentration protein solutions, while maintaining capacity for virus removal. This makes it highly suitable for large-volume production of biotherapeutic products.

Planova BioEX filters achieve high flow rates with reliable virus removal, thanks to a dense and homogeneous PVDF membrane produced by thermally induced phase separation. The PVDF membrane is made hydrophilic by graft polymerization.

Planova hollow fiber technology assures high quality consistency and provides outstanding scalability for biotherapeutic manufacturing.





## ■ **Effective for High-Pressure and High-Concentration Usage**

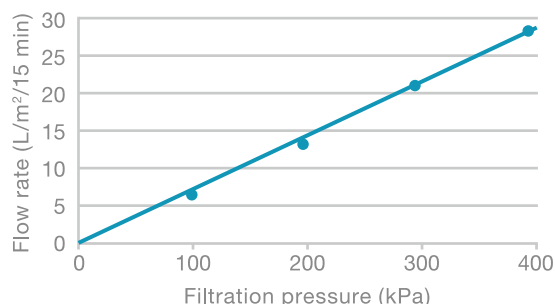
Research data supports the use of Planova BioEX filters for high-pressure, high-concentration filtrations.

Figure 5 shows the excellent correlation between filtration pressure and flow rate, which stays proportional even in the high pressure range.

Figure 6 shows that, with a 3% to 5% h-IgG solution, Planova BioEX maintains a high throughput of 6.5 kg/m<sup>2</sup>/3 hrs at filtration pressure of 294 kPa (42.6 psi).

Figure 5

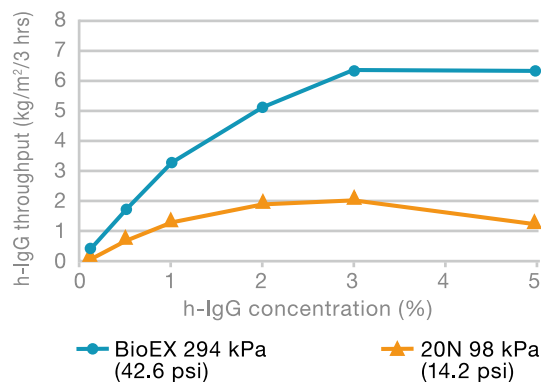
**Correlation between flow rate of 3% h-IgG solution and filtration pressure**



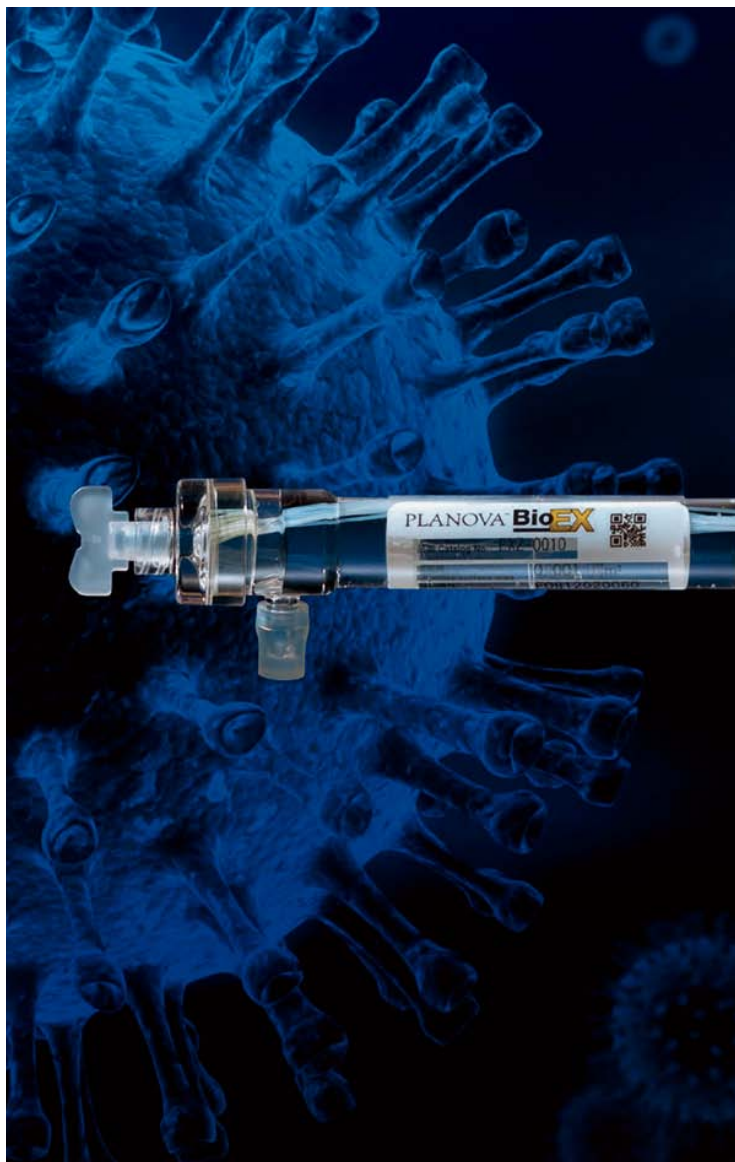
Flow rate is dependent on pressure. Higher pressure yields faster processing.

Figure 6

**Relationship between h-IgG throughput and h-IgG concentration**



Planova BioEX filters can withstand high-concentration solutions, thereby increasing the quantity of protein to be processed.



## ■ High LRV for Parvoviruses and Larger Viruses

Planova™ BioEX filters are able to remove parvoviruses, which are among the smallest known viruses found in nature, achieving levels below the detection threshold. Planova BioEX filters are validated to deliver > 4.0 logs of removal for PPV.

For high productivity, this performance is sustained even for large-volume filtration of high-concentration solutions, as shown in Figure 7.

Extensive testing of Planova BioEX filters validates performance for large viruses as well. For example, BioEX achieves > 5.5 logs for A-MuLV, as also shown in Figure 7.

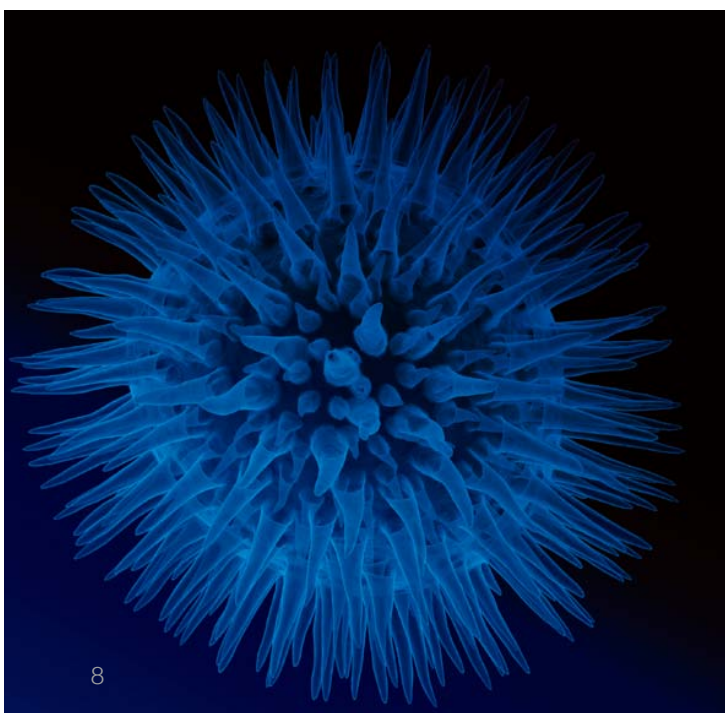
## ■ Laboratory-Scale and Process-Scale

In addition to the process-scale 4.0 m<sup>2</sup> and 1.0 m<sup>2</sup> Planova BioEX filter, the laboratory-scale 0.001 m<sup>2</sup> filter is offered for developing biological drug product manufacturing processes. Its smaller membrane surface area is convenient for scaled-down qualification or validation studies.

Additional Planova BioEX filter sizes, including 0.0003 m<sup>2</sup> and 0.1 m<sup>2</sup>, are available.

## ■ Standard Clamp Connections and SIP Capability

For easy integration into manufacturing processes, Planova BioEX 4.0 m<sup>2</sup>, 1.0 m<sup>2</sup> and 0.1 m<sup>2</sup> filters are designed for standard sanitary connections.





*Ferrule connection nozzle design enhances connection stability.*

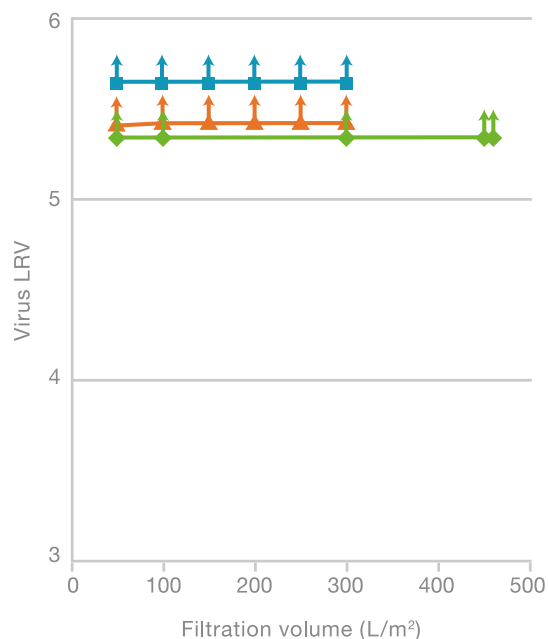
Membrane material and cartridge elements are constructed with the strength and integrity to withstand steam-in-place (SIP) operations.

#### ■ Predictable Results

Planova BioEX filters provide predictable performance under a considerable range of conditions, in many cases without prefilters.

Figure 7

#### Removability of PPV, MVM and A-MuLV for Planova BioEX



↑ Not detected

■ MVM

■ A-MuLV

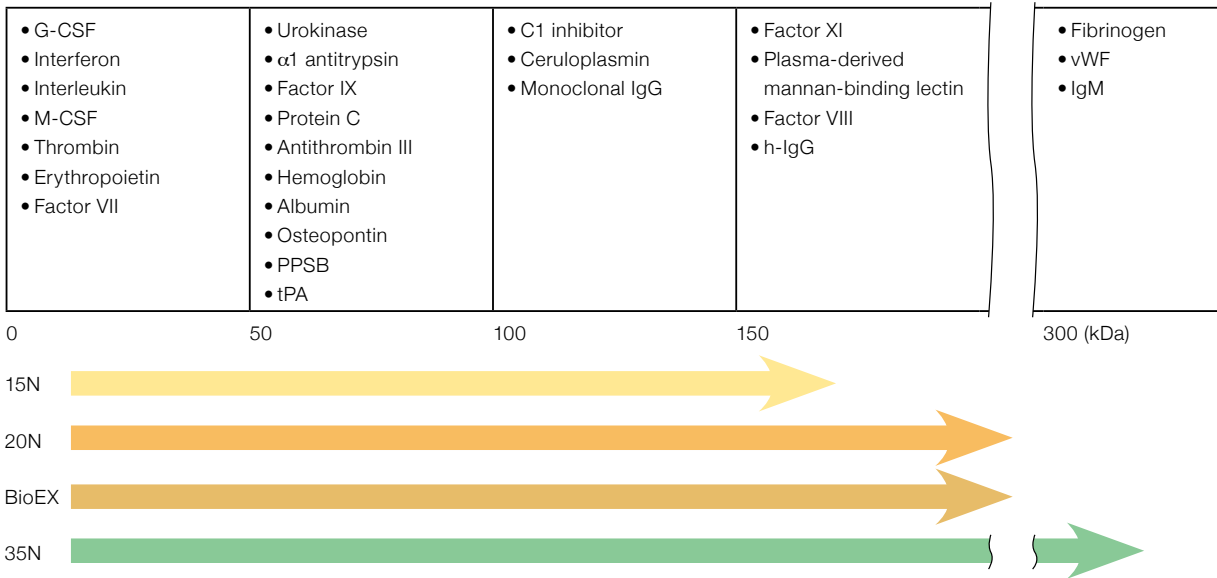
■ PPV

3% h-IgG was spiked with 0.5% by volume of each virus solution.  
Filtration Pressure: 294 kPa (42.6 psi)

*Even for high-volume filtration of high-concentration solutions, Planova BioEX removes parvoviruses and larger viruses.*



## Product Applications



## Examples of Protein Recovery Rate

	Polyclonal IgG (30mg/ml)	Monoclonal IgG (20mg/ml)	Factor VIII (100IU/ml)
Planova 15N	<90%	>95%	20-80%
Planova 20N	>95%	>98%	>85%
Planova 35N	100%	100%	>95%
Planova BioEX	>95%	>98%	NT

NT: Not tested

## Nomenclature

A-MuLV    amphotropic murine leukemia virus  
 B19        human parvovirus B19  
 BVDV      bovine viral diarrhea virus  
 CPV        canine parvovirus  
 EMCV      encephalomyocarditis virus  
 G-CSF      granulocyte-colony stimulating factor  
 HAV        hepatitis A virus  
 h-IgG       human immunoglobulin G  
 HIV        human immunodeficiency virus  
 HIV-1      human immunodeficiency virus type-1  
 HSV        herpes simplex virus  
 IgM        immunoglobulin M

M-CSF      macrophage-colony stimulating factor  
 Monoclonal IgG    monoclonal immunoglobulin G  
 MVM       minute virus of mice  
 Polio-1     poliovirus type 1  
 PPSB       prothrombin complex  
 PPV        porcine parvovirus  
 PRV        pseudorabies virus  
 Reo-3       reovirus type 3  
 SV40       simian virus 40  
 tPA        tissue plasminogen activator  
 vWF        von Willebrand factor  
 XMuLV     xenotropic murine leukemia virus



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Visit us on the web at [www.ak-bio.com](http://www.ak-bio.com)

### Warning

*Planova™ filters are not medical devices, and must not be used in transfusions, extracorporeal circulation, or other medical treatments.*

*Planova™ is a trademark of Asahi Kasei Medical Co., Ltd.*

**AsahiKASEI**



Pall Corporation

Contact Us: [www.pall.com/contact](http://www.pall.com/contact)

## Omega™ Ultrafiltration Membrane Disc Filters

### Description

#### High Performance and Stability for Users of Conventional, Non-Disposable Stirred Cells

- ▶ Polyethersulfone membrane provides higher flow rates and lower protein binding than competitive regenerated cellulose. This results in lower processing times and the highest possible recoveries.
- ▶ Discs can be washed and reused.



### Application

- ▶ Replacement membranes for other stirred cell systems
- ▶ Concentration, fractionation, purification, buffer exchange, or desalting of protein, cell broths, and other biomolecules

### Specifications

#### Filter Media

- ▶ Omega membrane (low protein-binding modified polyethersulfone)

#### Effective Filtration Area<sup>1</sup>

- ▶ 25 mm: 4.1 cm<sup>2</sup>
- ▶ 43 mm: 13.4 cm<sup>2</sup>
- ▶ 47 mm: 17.3 cm<sup>2</sup>
- ▶ 50 mm: 19.6 cm<sup>2</sup>
- ▶ 62 mm: 28.7 cm<sup>2</sup>
- ▶ 76 mm: 41.8 cm<sup>2</sup>
- ▶ 90 mm: 63 cm<sup>2</sup>
- ▶ 150 mm: 162 cm<sup>2</sup>

#### pH Range

- ▶ 1 - 14

#### Operating Temperature Range

- ▶ 0 - 40°C (32 - 104 °F)

#### Sanitization

- ▶ Provided non-sterile. May be sanitized using 70% ethanol or 200 ppm sodium hypochlorite.

<sup>1</sup>Actual effective filtration area is dependent on the filter holder used.

### Performance

#### Deionized Water and Solute Flow Rates

Filter Type	Water Flux <sup>2</sup> (mL/min/cm <sup>2</sup> )	Solute Flux (mL/min/cm <sup>2</sup> )	Solute Type
1K	0.01 - 0.1	0.01 - 0.08	Bacitracin

3K	0.1 - 0.25	0.06 - 0.2	Albumin <sup>3</sup>
5K	0.25 - 0.5	0.18 - 0.21	Albumin
10K	0.7 - 1.9	0.2 - 0.32	Albumin
30K	1.9 - 4	0.21 - 0.26	Albumin
50K	2.3 - 4.5	0.23 - 0.28	Albumin
100K	5 - 14	1.5 - 6.5	Albumin
300K <sup>4</sup>	1 - 3	0.4 - 3	Albumin

<sup>2</sup>Deionized water at 25 °C (77 °F) and 3.7 bar (370 kPa, 55 psi)

<sup>3</sup>0.2% saline buffer solution at 25 °C (77 °F) and 3.7 bar (370 kPa, 55 psi)

<sup>4</sup>0.7 bar (70 kPa, 10 psi)

Data derived using Omega membrane stirred cells. When using tangential cassette systems, solute fluxes will be higher and water fluxes will be lower.

## Ordering Information

Part Number	Description	Pkg	Price	Qty
-------------	-------------	-----	-------	-----

### Omega Membrane Discs Filters (25, 43, 47, 50, 62, and 76 mm provided 12/pkg; 90 and 150 mm provided 6/pkg)

#### Omega Membrane Discs - 1K

OM001025	25 mm	12/pkg	NA	<input type="text" value="0"/>
OM001043	43 mm	12/pkg	NA	<input type="text" value="0"/>
OM001047	47 mm	12/pkg	NA	<input type="text" value="0"/>
OM001050	50 mm	12/pkg	N/A	<input type="text" value="0"/>
OM001062	62 mm	12/pkg	NA	<input type="text" value="0"/>
OM001076	76 mm	12/pkg	NA	<input type="text" value="0"/>
OM001090	90 mm	6/pkg	NA	<input type="text" value="0"/>
OM001150	150 mm	6/pkg	NA	<input type="text" value="0"/>

#### Omega Membrane Discs - 3K

OM003025	25 mm	12/pkg	NA	<input type="text" value="0"/>
OM003043	43 mm	12/pkg	NA	<input type="text" value="0"/>
OM003047	47 mm	12/pkg	NA	<input type="text" value="0"/>
OM003050	50 mm	12/pkg	NA	<input type="text" value="0"/>
OM003062	62 mm	12/pkg	NA	<input type="text" value="0"/>
OM003076	76 mm	12/pkg	NA	<input type="text" value="0"/>
OM003090	90 mm	6/pkg	NA	<input type="text" value="0"/>
OM003150	150 mm	6/pkg	NA	<input type="text" value="0"/>

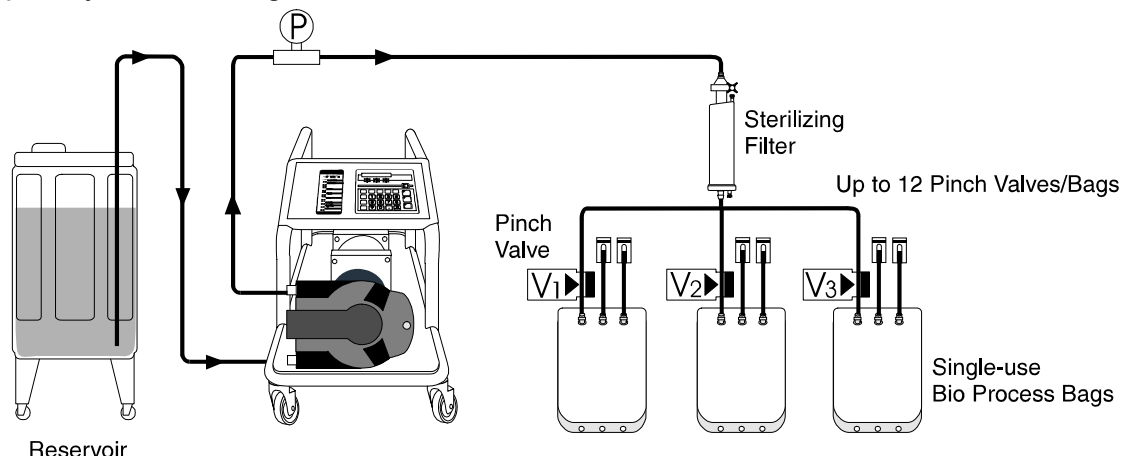
#### Omega Membrane Discs - 5K

OM005025	25 mm	12/pkg	NA	<input type="text" value="0"/>
OM005043	43 mm	12/pkg	NA	<input type="text" value="0"/>
OM005047	47 mm	12/pkg	NA	<input type="text" value="0"/>

## Automated, Aseptic Transfer of Liquids into Multiple, Single-use Storage Bags

### Summary:

The Fill Master aseptically transfers solution into multiple, plastic storage bags. The Fill Master controls up to twelve, pneumatically operated tube pinch valves to direct the liquid flow from the reservoir into sterile storage bags. Only the internal tube walls and the inside bag surfaces are in contact with the liquid. The high-accuracy, low-shear peristaltic pump action provides gentle and reproducible bag filling. In the programmable, volume dispensing mode, the Fill Master meters a user-defined volume of liquid, then automatically switches to the next empty storage bag to be filled. Up to 12 storage bags can be serially filled in this fashion. Once filled, the bags can be aseptically sealed using a sterile tube welder.



### Features:

There are several Fill Master models to choose from. For example, the Fill Master 104B peristaltic pump head (max. pump rate is 14.6 liters/min with #184 Silicone tubing) allows for a continuous tube connection to be made between the reservoir and the storage bag manifold. A typical sterile fluid handling bag manifold, which may include multiple storage bags, as well as integral tubing, QC bags, sterilizing filter and connectors, are available pre-sterilized (i.e. gamma irradiated). Contact SciLog for info.

When ordering a sterile fluid handling bag manifold, the size and material of the integral tubing must be specified. The Fill Master 104B peristaltic pump head can accommodate the following tubing sizes: #26, 6.4 mm ID (0.15 to 2.60 liters/min); #73, 9.6mm ID (0.31 to 5.94 liters/min); #82, 12.7 mm ID (0.53 to 9.25 liters/min) and #184, 15.9 mm ID (0.80 to 14.50 liters/min) A range of tube materials can be used: Silicone, Pharmed, PVC and C-Flex (heat sealable). Other Fill Master models accommodate additional sizes.

An optional, disposable pressure transmitter can be placed in front of the in-line sterilizing filter to monitor filter backpressure. With the pressure transmitter connected to the Fill Master, excessive pressure build-up as well as associated leaks and bag failures are prevented. The Fill Master will stop all pumping action when a user-defined safe pressure limit is exceeded.

## Automated Bag Filling Operation: *Fill Master Metering Program*

The Fill Master Volume-Flow mode (Mode 30) allows you to enter (in "EDIT") and store an automated metering program. The following is a simple program to fill three, 20 liter storage bags. Change the RATE and TIME program steps to suit your storage bag volume. Add additional "RUN" program blocks to increase the number of bags (up to 12) you want to fill.

000	<b>START</b>	<i>The following program steps have been entered in Mode 30:</i>
001	<b>CW</b>	<i>Motor Runs Clock-wise</i>
002	<b>RUN</b>	<i>Motor is turned "ON"</i>
003	<b>V 100000</b>	<i>Pinch Valve V1 is Energized, other V-valves are De-energized</i>
004	<b>RATE: 5.0L/min</b>	<i>Pump Rate 5 liters per minute</i>
005	<b>TIME: 00:04:00</b>	<i>Pump Runs 4 minutes, <b>Bag #1</b> is filled with 20 Liters</i>
006	<b>STOP</b>	<i>Pump "Off"</i>
007	<b>V 020000</b>	<i>Pinch Valve V2 is Energized, other V-valve are De-energized</i>
008	<b>TIME: 00:00:02</b>	<i>2 Second Time delay</i>
009	<b>RUN</b>	<i>Pump "ON"</i>
010	<b>RATE: 5.0 L/min</b>	<i>Pump Rate 5 liters per minute</i>
011	<b>TIME: 00:04:00</b>	<i>Pump Runs 4 Minutes, <b>Bag #2</b> is filled with 20 Liters</i>
012	<b>STOP</b>	<i>Pump "Off"</i>
013	<b>V 003000</b>	<i>Pinch Valve V3 is Energized, other V-valves are De-energized</i>
014	<b>TIME: 00:00:02</b>	<i>2 Second Time Delay</i>
015	<b>RUN</b>	<i>Pump "ON"</i>
016	<b>RATE: 5.0 L/min</b>	<i>Pump Rate 5.0 liters per minute</i>
017	<b>TIME: 00:04:00</b>	<i>Pump Runs 4 Minutes, <b>Bag #3</b> is filled with 20 Liters</i>
018	<b>STOP</b>	<i>Pump "Off"</i>
019	<b>V 000000</b>	<i>All V-Valves are De-energized</i>
020	<b>COUNT: 1</b>	<i>The Program Steps 000 to 020 are executed once</i>
021	<b>END</b>	

## ORDERING INFORMATION:

**SciLog Customer Service: 1-800-955-1993**

### Catalog #: Description:

- 800-104BFILL** **Fill Master 104B mounted with a Watson-Marlow 620R peristaltic pump head**, 0.5 HP motor; 90VDC; 250 RPM max.; 7.8: 1 Speed Reducer. The 620R peristaltic pump head has two (2) pump rollers and accommodates continuous pump tubing of the following sizes: 6.4mm ID (#26), 0.15 to 2.60 L/M; 9.6mm ID (#73) 0.31 to 5.94 L/M; 12.7mm ID (#82), 0.53 to 9.25 L/M; and 15.9mm ID (#184), 0.80 to 14.5 L/M, wall thickness is 3.2mm (0.125") for all tube sizes
- 800-450** **Multiple-Valve Controller:** Remote control of twelve (12) electrically actuated air flow valves (Clippard) for automated pneumatic operation of twelve (12) pinch valves or sanitary diaphragm valves. Fully programmable from the Fill Master via dedicated serial port or manual control from front panel of the multi-Valve Box. Optional inputs for valve position sensors.
- 934-VALVE** **Tubing Pinch Valve:** Specify Tubing OD: 0.125", 0.250", 0.375", 0.50", 0.75", and wall thickness. The valve is pneumatically actuated. Ideal for maintaining sterility, only tubing comes in contact with your process solution. Requires a Multi-Valve Controller for actuation and control. The Multi-Valve Controller is fully programmable from the Fill Master via dedicated serial port or manual control from front panel of multi-Valve Box. Optional inputs for valve position sensors.

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# Labtainer™ System

BioProcess Container™ Systems for small volume liquid handling applications in bioresearch and biopharma production.

*Animal Derived Component Free (ADCF™) Film • Sizes range from 50 mL–50 L*

.....

## Applications:

- Collection, storage, and transport of sterile liquids
- Delivery of media to small-scale biotechnology production systems
- Formulation and filling of sterile media, buffers, and other process liquids
- Chromatography feed and fraction collection
- Bioreactor and fermentation feed and harvest
- Transportation and storage of bulk intermediate product
- Freezing/thawing, heat inactivation, and irradiation of product

## HyQ®CX5-14 Film

- Regulatory friendly due to ADCF status and low extractables
- Improved shelf life of media due to superior barrier properties
- Scalability from 50 mL–2500 L in same film

## Flexible and Secure Containers

- Save storage space
- Safer than carboys
- Easy to handle (handling systems available)

## Single-Use; Arrive Sterile and Ready-to-use

- No cleaning or sterilization
- Minimize validation requirements
- Labor and cost saving

## Range of Standard Products

- Integrate with most industry standard connection systems







## B I O - P H A R M

# CIP Washers

## Clean-In-Place Systems

Sani-Matic Clean-In-Place Systems for the Bio-Pharm industry are custom engineered to specific plant application and utility requirements to ensure effective and efficient cleaning of process equipment automatically.

Proper CIP design and sizing will not only ensure sufficient flow and pressure to remove residue adequately, and rinse thoroughly. Sani-Matic CIP designs will also save cycle time, reduce water and chemical usage, and minimize discharge and utility costs. Balancing all factors to create an optimum system is our goal. CIP Systems can be designed to recirculate or provide "once-through" cleaning, depending on the product residue. Multi-tank systems can provide additional benefits of faster turnaround time if cleaning processes are more frequent.

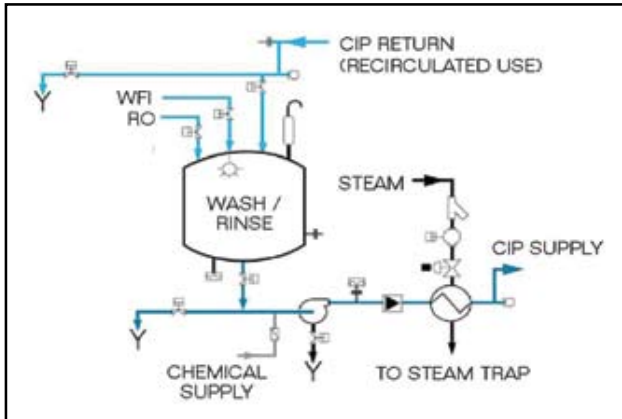
### WHAT MAKES US DIFFERENT?

Sani-Matic has the expertise to engineer an effective solution for your cleaning application. Specialists in cleaning technology, the Sani-Matic team will provide valuable insight and guidance during your project from beginning to end. Developing a creative design concept based on decades of practical experience, we can ensure you are purchasing a system which is dependable and cost-effective for the long term. Sani-Matic has in-house programming staff and field technical service with the expertise to develop and optimize your cleaning cycles and integrate the CIP functions with your plant Process control systems. Because the Sani-Matic team understands the unique challenges of cleaning, we add value to your CIP projects.



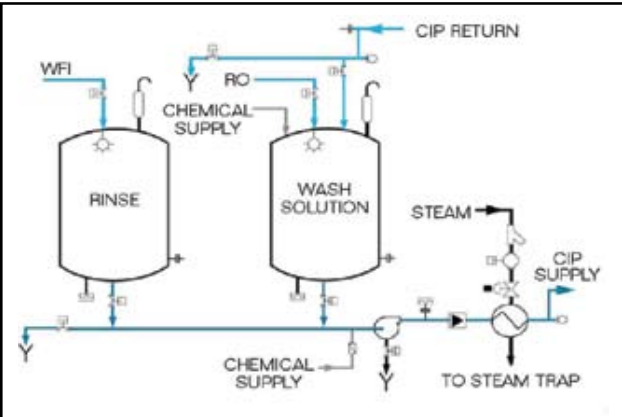
Our manufacturing facilities and processes are controlled and efficient, ensuring that your system is compliant and on-schedule. Project management and documentation is detailed and complete, saving you time and resources while achieving your project's budget and target start-up date.

## One Tank Single-use System



- Single-use source of wash solution and rinse water
- Lower space requirement
- Portable or stationary design is available
- Once through or recirculated

## Two Tank Detergent & Rinse System



- Permits once through or recirculated flow of wash solution
- Used where water utilities are limited
- Reduced wash cycle times

### CIP DESIGN SIZING FACTORS

CIP system sizing is determined by dimensions of the vessels and pipelines to be cleaned.

Supply pump flow is determined by the vessel size and the largest pipeline diameter. Turbulent cleaning action requires 5-7 fps flow in lines. Proper cascading cleaning action by a sprayball is 3 gpm per ft of tank circumference.

Pressure is determined by the spray device requirement (typically 25psi, plus any line loss).

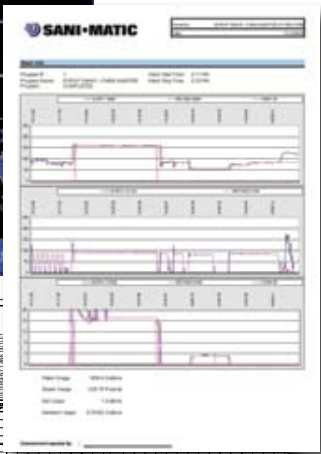
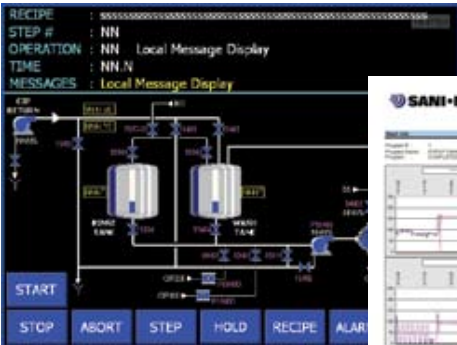
Sizing the wash and rinse tank volume is determined by the pipeline holdup volume (gal per 100ft) and the available inlet water capacity and the estimated cleaning program time.

Tube OD	Tube ID	Volume Per 100'	Industry Minimum 5 ft/sec	Recommended 7 ft/sec	Maximum Gravity Flow thru Outlet
0.5"	0.37"	0.56 gal	2 GPM	3 GPM	
0.75"	0.62"	1.57 gal	5 GPM	7 GPM	
1.0"	0.87"	3.1 gal	10 GPM	13 GPM	
1.5"	1.37"	7.7 gal	23 GPM	32 GPM	35-40 GPM
2.0"	1.87"	14 gal	43 GPM	60 GPM	75-80 GPM
2.5"	2.37"	23 gal	69 GPM	96 GPM	115-120 GPM
3.0"	2.87"	34 gal	101 GPM	141 GPM	190-200 GPM
4.0"	3.834"	60 gal	180 GPM	252 GPM	250-275 GPM
6.0"	5.834"	139 gal	417 GPM	583 GPM	720 GPM



# Controls

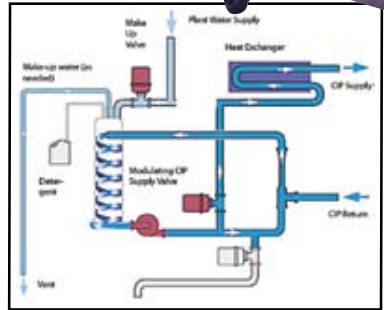
## Easier/Faster to Validate



OPCODE	DESCRIPTION	OPCODE	DESCRIPTION
1	"PROGRAM END"	11	"CHASE RINSE TO DRAIN"
2	"SAN PROGRAM START"	12	"BURST PRE RINSE TO DRAIN"
3	"CIP PROGRAM END"	13	"DETERGENT WASH"
4	"PRE RINSE TO DRAIN"	14	"ACID WASH"
5	"BURST PRE RINSE TO DRAIN"	15	"CHASE RINSE TO DRAIN W/ DETERGENT"
6	"DETERGENT WASH"	16	"CHASE RINSE TO DRAIN W/ ACID AND CONDUCTIVITY HOLD"
7	"ACID WASH"	17	"CHEMICAL SANITIZE"
8	"CHASE RINSE TO DRAIN W/ DETERGENT"	18	"RINSE TO DRAIN"
9	"CHASE RINSE TO DRAIN W/ ACID AND CONDUCTIVITY HOLD"	19	"RINSE TO DRAIN WITH DEIONIZED CONDUCTIVITY HOLD"
10	"CHEMICAL SANITIZE"	20	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
11	"RINSE TO DRAIN"	21	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
12	"RINSE TO DRAIN WITH DEIONIZED CONDUCTIVITY HOLD"	22	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
13	"BURST PRE RINSE TO DRAIN"	23	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
14	"DETERGENT WASH"	24	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
15	"ACID WASH"	25	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
16	"CHASE RINSE TO DRAIN W/ DETERGENT"	26	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
17	"CHASE RINSE TO DRAIN W/ ACID AND CONDUCTIVITY HOLD"	27	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
18	"CHEMICAL SANITIZE"	28	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
19	"RINSE TO DRAIN"	29	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"
20	"RINSE TO DRAIN WITH DEIONIZED CONDUCTIVITY HOLD"		
21	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"		
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27	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"		
28	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"		
29	"RINSE WITH ACID RECOVERY AND RETURN CONDUCTIVITY HOLD"		

# UltraFlow

UltraFlow technical brochure available separately.



Sani-Matic has in-house programmers and authorized UL panel shop who design, manufacture, pre-test, startup and support of all types of Control Systems for cleaning systems. Because the team specializes in cleaning applications, Sani-Matic has developed expertise and efficiencies that have advantages over other fabricators or process integrators. Sani-Matic has designed, manufactured and supported thousands of CIP systems and other cleaning related systems.

Understanding the unique aspects of an effective cleaning program, Sani-Matic developed a very flexible & easy-to-understand OP-Code Recipe Editor that allows the customer to manipulate the system hardware to optimize their cleaning programs. This can result shorter total cycle time, lower water & chemical usage, more precise control to setpoints and detailed alarms which reduce troubleshooting time.

Soon to be released, the new Sani-Trend Data Acquisition System collects and stores cleaning cycle data, events/alarms, and operator information onto a PC. Operating usage of water, chemical, and utilities may be calculated and trended. Sani-Trend reports are in easy to use Excel format, and provide reliable, secure information giving you valuable insight on your cleaning process.

The Sani-Matic UltraFlow CIP is patented technology which uses an eductor, vortex air separation chamber and modulating valves to control the CIP supply and return flows. This unique CIP design successfully brings air & water back in the return flow, separates the air, keeping the supply pump primed. The UltraFlow uses much less water than traditional CIPs and is flexible to clean a wide variety of circuits.

## ADVANTAGES

- Reduce water usage
- Lower chemical consumption
- Eliminate installation costs
- Increase flexibility

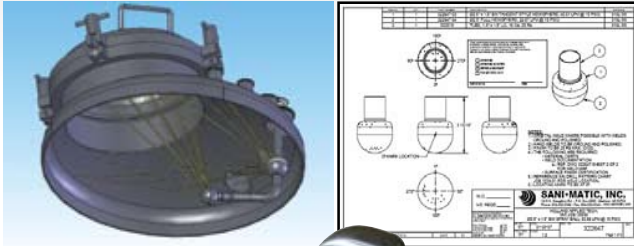
## THE IDEAL SOLUTION FOR:

- Vessels with low and/or small outlets
- Variable supply and return flow (1 to 100 GPM)
- Portable - Avoid investment in permanent supply & return lines
- Locations with limited/low water volume available
- Economical replacement for an existing or outdated CIP
- Installations where available floor space is limited
- Not appropriate for "once through" non-recirculated processes

# CIP Features

## Spray Devices

### Custom Engineered and Precision Drilled



- Custom engineered in 3D
- Precision drilled
- Pass Riboflavin testing first time
- Documented for future replacement without re-validating

Sani-Matic designs and manufactures a complete range of sprayballs and associated solution tubes and tank fittings.

Utilizing the latest technology, Sani-Matic has engineered a method to model a process vessel in 3D and design the most effective spray device and drill pattern to ensure proper coverage of all ports and surfaces. With decades of experience in spray technology and Bio-Pharm CIP applications, the Sani-Matic team understands the spray dynamics required to ensure adequate flows, pressures and geometries will dependably clean your process equipment.

Sani-Matic provides complete documentation and ID marking for ease of validation and future replacement. Responsive service and reliable delivery make Sani-Matic the preferred supplier of Spray Devices in the market today.

## Documentation

### Faster & Easier Validation

- Operation and maintenance manuals
- Recommended spare parts list
- Instrument lists
- Instrumentation calibration procedures
- Performance data
- Material certificates
- Weld qualification and inspection records
- Inspection test results, reports and certificates
- ASME data
- Component catalog cut sheets
- As built assembly drawings
- As built process and instrumentation diagrams
- As built electrical drawings
- Annotated PLC ladder diagrams

#### OPTIONAL

- (FRS/FDS) Functional Design Specifications
- Control System Design Specification (HRS and SRS)
- (FAT) Factory Acceptance Test report
- (SAT) Site Acceptance Test document
- IQ/OQ installation and operation qualification
- Cleaning and passivation report
- Weld video record (Boroscope)

Sani-Matic personnel are active participants in the following organizations:

*ISPE-International Society of Pharmaceutical Engineers*

*Co-developer & Co-leader of "Cleaning Technology" course*

*ASME-American Society of Mechanical Engineers*

*BPE (Bio Processing Equipment) CIP task group*

*AWS-American Welding Society*

*D18 Team committee for sanitary welding*

*3A-Sanitary Standards*

*Member of board of directors and task committees*

*In-house engineering and operations*

*Custom engineered to order – solidworks 3D Cadd designs*

*Electrical design and programming in-house*

*Manufacturing work team dedicated to Bio-Pharm*

*Authorized UL panel shop*

*ASME certified shop – welding inspector and trainer*

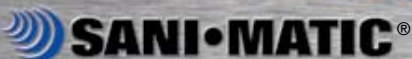
*Project Management – manage Gantt scheduling, change orders, FAT*

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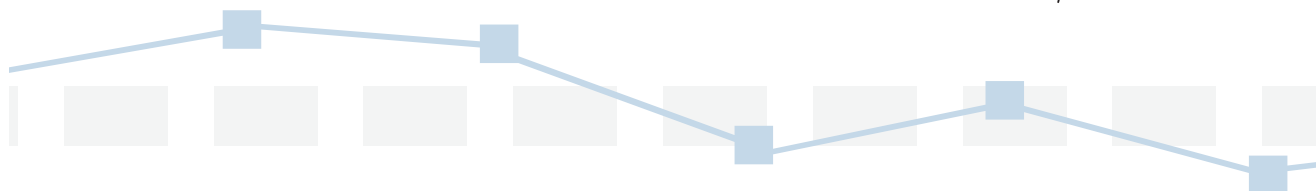
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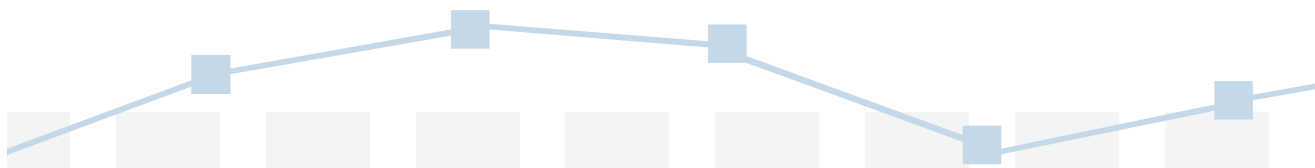


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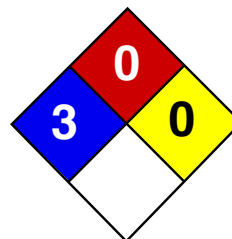
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## **Appendix I: MSDS Sheets**



Health	3
Fire	0
Reactivity	0
Personal Protection	

## Material Safety Data Sheet

### Phosphoric acid, 85% MSDS

#### Section 1: Chemical Product and Company Identification

**Product Name:** Phosphoric acid, 85%

**Catalog Codes:** SLP5569, SLP4555, SLP1732

**CAS#:** Mixture.

**RTECS:** Not applicable.

**TSCA:** TSCA 8(b) inventory: Phosphoric Acid; Water

**CI#:** Not available.

**Synonym:** Phosphoric Acid 85%; Phosphoric Acid; Orthophosphoric acid

**Chemical Name:** Not applicable.

**Chemical Formula:** Not applicable.

#### Contact Information:

**Sciencelab.com, Inc.**

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Houston, Texas 77396

US Sales: **1-800-901-7247**

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**CHEMTREC (24HR Emergency Telephone), call:**

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**International CHEMTREC, call:** 1-703-527-3887

**For non-emergency assistance, call:** 1-281-441-4400

#### Section 2: Composition and Information on Ingredients

##### Composition:

Name	CAS #	% by Weight
Phosphoric Acid	7664-38-2	85-88
Water	7732-18-5	12-15

**Toxicological Data on Ingredients:** Phosphoric Acid: ORAL (LD50): Acute: 1530 mg/kg [Rat]. DERMAL (LD50): Acute: 2740 mg/kg [Rabbit]. DUST (LC50): Acute: >850 mg/m 1 hours [Rat].

#### Section 3: Hazards Identification

##### Potential Acute Health Effects:

Very hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, . Hazardous in case of skin contact (corrosive, permeator), of eye contact (corrosive). Slightly hazardous in case of inhalation (lung sensitizer). Liquid or spray mist may produce tissue damage particularly on mucous membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

##### Potential Chronic Health Effects:

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance may be toxic to blood, liver, skin, eyes, bone marrow. Repeated



or prolonged exposure to the substance can produce target organs damage. Repeated or prolonged contact with spray mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to spray mist may produce respiratory tract irritation leading to frequent attacks of bronchial infection. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

#### Section 4: First Aid Measures

**Eye Contact:**

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

**Skin Contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

**Serious Skin Contact:**

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

**Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

**Serious Inhalation:**

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. **WARNING:** It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

**Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

#### Section 5: Fire and Explosion Data

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** of metals

**Explosion Hazards in Presence of Various Substances:** Non-explosive in presence of open flames and sparks, of shocks.

**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:**

Reacts with metals to liberate flammable hydrogen gas. Formation of flammable gases with aldehydes, cyanides, mercaptans, and sulfides.

**Special Remarks on Explosion Hazards:** Mixtures with nitromethane are explosive. (Phosphoric Acid)

#### Section 6: Accidental Release Measures

**Small Spill:**

Dilute with water and mop up, or absorb with an inert dry material and place in an appropriate waste disposal container. If necessary: Neutralize the residue with a dilute solution of sodium carbonate.

**Large Spill:**

Corrosive liquid. Poisonous liquid. Stop leak if without risk. Absorb with DRY earth, sand or other non-combustible material. Do not get water inside container. Do not touch spilled material. Use water spray curtain to divert vapor drift. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of sodium carbonate. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

## Section 7: Handling and Storage

**Precautions:**

Do not ingest. Do not breathe gas/fumes/ vapor/spray. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, combustible materials, metals, alkalis. May corrode metallic surfaces. Store in a metallic or coated fiberboard drum using a strong polyethylene inner package.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area.

## Section 8: Exposure Controls/Personal Protection

**Engineering Controls:**

Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

**Personal Protection:**

Face shield. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Gloves. Boots.

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Vapor respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:**

Phosphoric Acid TWA: 1 STEL: 3 (mg/m<sup>3</sup>) from ACGIH (TLV) [United States] TWA: 1 STEL: 3 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] TWA: 1 STEL: 3 (mg/m<sup>3</sup>) from NIOSH TWA: 1 STEL: 3 (mg/m<sup>3</sup>) [Mexico] Consult local authorities for acceptable exposure limits.

## Section 9: Physical and Chemical Properties

**Physical state and appearance:** Liquid. (Syrupy liquid Viscous liquid.)

**Odor:** Odorless.

**Taste:** Acid.

**Molecular Weight:** Not applicable.

**Color:** Clear Colorless.

**pH (1% soln/water):** Acidic.

**Boiling Point:** 158°C (316.4°F)

**Melting Point:** 21°C (69.8°F)

**Critical Temperature:** Not available.

**Specific Gravity:** 1.685 @ 25 C (Water = 1)

**Vapor Pressure:** 0.3 kPa (@ 20°C)

**Vapor Density:** 3.4 (Air = 1)

**Volatility:** Not available.

**Odor Threshold:** Not available.

**Water/Oil Dist. Coeff.:** Not available.

**Ionicity (in Water):** Not available.

**Dispersion Properties:** See solubility in water.

**Solubility:**

Easily soluble in hot water. Soluble in cold water.

## Section 10: Stability and Reactivity Data

**Stability:** The product is stable.

**Instability Temperature:** Not available.

**Conditions of Instability:** Incompatible materials

**Incompatibility with various substances:** Reactive with oxidizing agents, combustible materials, metals, alkalis.

**Corrosivity:**

Extremely corrosive in presence of copper, of stainless steel(304), of stainless steel(316). Highly corrosive in presence of aluminum. Non-corrosive in presence of glass.

**Special Remarks on Reactivity:**

Reacts with metals to liberate flammable hydrogen gas. Incompatible with sodium tetrahydroborate producing a violent exothermic reaction. Heat generated with: alcohols, glycols, aldehydes, amides, amines, azo-compounds, carbamates, caustics, esters, ketones, phenols and cresols, organophosphates, epoxides, combustible materials, unsaturated halides, organic peroxides. Formation of flammable gases, with aldehydes, cyanides, mercaptins, and sulfides. Formation of toxic fumes with cyanides, fluorides, halogenated organics, sulfides, and organic peroxides. Do not mix with solutions containing bleach or ammonia. Incompatible with nitromethane, chlorides + stainless steel. (Phosphoric Acid)

**Special Remarks on Corrosivity:**

Minor corrosive effect on bronze. Severe corrosive effect on brass. Corrosive to ferrous metals and alloys.

**Polymerization:** Will not occur.

## Section 11: Toxicological Information

**Routes of Entry:** Absorbed through skin. Dermal contact. Eye contact. Inhalation. Ingestion.

**Toxicity to Animals:**

Acute oral toxicity (LD50): 1530 mg/kg [Rat]. Acute dermal toxicity (LD50): 2740 mg/kg [Rabbit].

**Chronic Effects on Humans:** May cause damage to the following organs: blood, liver, skin, eyes, bone marrow.

**Other Toxic Effects on Humans:**

Extremely hazardous in case of inhalation (lung corrosive). Very hazardous in case of skin contact (irritant), of ingestion, . Hazardous in case of skin contact (corrosive, permeator), of eye contact (corrosive).

**Special Remarks on Toxicity to Animals:** Not available.

**Special Remarks on Chronic Effects on Humans:** Not available.

**Special Remarks on other Toxic Effects on Humans:**

Acute Potential Health Effects: Skin: Corrosive and causes severe skin irritation and can cause severe skin burns. May affect behavior (somnolence or excitement) if absorbed through skin. Eyes: Corrosive. Liquid or vapor causes severe eye irritation and can cause severe eye burns leading to permanent corneal damage or chemical conjunctivitis. Ingestion: May be harmful if swallowed. Causes irritation and burns of the gastrointestinal (digestive) tract. Causes severe pain, nausea, vomiting, diarrhea hematemesis, gastrointestinal hemorrhaging, and shock. May cause corrosion and permanent tissue destruction of the esophagus and digestive tract. May affect behavior and urinary system, liver (hepatocellular damage, hepatic enzymes increased), blood (blood dyscrasia). May also

## Section 12: Ecological Information

**Ecotoxicity:** Not available.

**BOD5 and COD:** Not available.

**Products of Biodegradation:**

Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

**Toxicity of the Products of Biodegradation:** The products of degradation are less toxic than the product itself.

**Special Remarks on the Products of Biodegradation:** Not available.

## Section 13: Disposal Considerations

**Waste Disposal:**

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

## Section 14: Transport Information

**DOT Classification:** Class 8: Corrosive material

**Identification:** : Phosphoric acid (Phosphoric Acid) UNNA: 1805 PG: III

**Special Provisions for Transport:** Not available.

## Section 15: Other Regulatory Information

**Federal and State Regulations:**

Connecticut hazardous material survey.: Phosphoric Acid Illinois toxic substances disclosure to employee act: Phosphoric acid Illinois chemical safety act: Phosphoric acid New York release reporting list: Phosphoric acid Rhode Island RTK hazardous substances: Phosphoric acid Pennsylvania RTK: Phosphoric acid Minnesota: Phosphoric acid Massachusetts RTK: Phosphoric acid Massachusetts spill list: Phosphoric acid New Jersey: Phosphoric acid New Jersey spill list: Phosphoric acid Louisiana spill reporting: Phosphoric acid California Director's list of hazardous substances: Phosphoric acid TSCA 8(b) inventory: Phosphoric Acid; Water SARA 313 toxic chemical notification and release reporting: Phosphoric acid CERCLA: Hazardous substances.: Phosphoric acid: 5000 lbs. (2268 kg)

**Other Regulations:** OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).

**Other Classifications:**

**WHMIS (Canada):** CLASS E: Corrosive liquid.

**DSCL (EEC):**

R34- Causes burns. S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**HMIS (U.S.A.):**

**Health Hazard:** 3

**Fire Hazard:** 0

**Reactivity:** 0

**Personal Protection:**

**National Fire Protection Association (U.S.A.):**

**Health:** 3

**Flammability:** 0

**Reactivity:** 0

**Specific hazard:**

**Protective Equipment:**

Gloves. Full suit. Vapor respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate. Face shield.

## Section 16: Other Information

**References:** Not available.

**Other Special Considerations:** Not available.

**Created:** 10/10/2005 08:47 PM

**Last Updated:** 05/21/2013 12:00 PM

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## SAFETY DATA SHEET

Version 4.9  
Revision Date 04/05/2014  
Print Date 04/10/2014

---

**1. PRODUCT AND COMPANY IDENTIFICATION****1.1 Product identifiers**

Product name : EX-CELL® ACF CHO Medium without L-glutamine,  
animal component free

Product Number : C9098  
Brand : Sigma  
REACH No. : A registration number is not available for this substance as the substance  
or its uses are exempted from registration, the annual tonnage does not  
require a registration or the registration is envisaged for a later  
registration deadline.

**1.2 Relevant identified uses of the substance or mixture and uses advised against**

Identified uses : Laboratory chemicals, Manufacture of substances

**1.3 Details of the supplier of the safety data sheet**

Company : Sigma-Aldrich  
3050 Spruce Street  
SAINT LOUIS MO 63103  
USA

Telephone : +1 800-325-5832  
Fax : +1 800-325-5052

**1.4 Emergency telephone number**

Emergency Phone # : (314) 776-6555

---

**2. HAZARDS IDENTIFICATION****2.1 Classification of the substance or mixture**

Not a hazardous substance or mixture.

**2.2 GHS Label elements, including precautionary statements**

Not a hazardous substance or mixture.

**2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none**

---

**3. COMPOSITION/INFORMATION ON INGREDIENTS****3.2 Mixtures**

Synonyms : CHO Medium

No ingredients are hazardous according to OSHA criteria.  
No components need to be disclosed according to the applicable regulations.

---

**4. FIRST AID MEASURES****4.1 Description of first aid measures****If inhaled**

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

**In case of skin contact**

Wash off with soap and plenty of water.

**In case of eye contact**

Flush eyes with water as a precaution.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water.

**4.2 Most important symptoms and effects, both acute and delayed**

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

**4.3 Indication of any immediate medical attention and special treatment needed**

no data available

---

**5. FIREFIGHTING MEASURES****5.1 Extinguishing media****Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**5.2 Special hazards arising from the substance or mixture**

Nature of decomposition products not known.

**5.3 Advice for firefighters**

Wear self contained breathing apparatus for fire fighting if necessary.

**5.4 Further information**

no data available

---

**6. ACCIDENTAL RELEASE MEASURES****6.1 Personal precautions, protective equipment and emergency procedures**

Avoid dust formation. Avoid breathing vapours, mist or gas.

For personal protection see section 8.

**6.2 Environmental precautions**

Do not let product enter drains.

**6.3 Methods and materials for containment and cleaning up**

Sweep up and shovel. Keep in suitable, closed containers for disposal.

**6.4 Reference to other sections**

For disposal see section 13.

---

**7. HANDLING AND STORAGE****7.1 Precautions for safe handling**

Provide appropriate exhaust ventilation at places where dust is formed.

For precautions see section 2.2.

**7.2 Conditions for safe storage, including any incompatibilities**

Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

**7.3 Specific end use(s)**

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

---

**8. EXPOSURE CONTROLS/PERSONAL PROTECTION****8.1 Control parameters****Components with workplace control parameters**

Contains no substances with occupational exposure limit values.

**8.2 Exposure controls****Appropriate engineering controls**

General industrial hygiene practice.

## Personal protective equipment

### Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

### Skin protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

### Body Protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

### Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

### Control of environmental exposure

Do not let product enter drains.

---

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### 9.1 Information on basic physical and chemical properties

a) Appearance	Form: powder
b) Odour	no data available
c) Odour Threshold	no data available
d) pH	no data available
e) Melting point/freezing point	no data available
f) Initial boiling point and boiling range	no data available
g) Flash point	no data available
h) Evaporation rate	no data available
i) Flammability (solid, gas)	no data available
j) Upper/lower flammability or explosive limits	no data available
k) Vapour pressure	no data available
l) Vapour density	no data available
m) Relative density	no data available
n) Water solubility	no data available
o) Partition coefficient: n-octanol/water	no data available
p) Auto-ignition temperature	no data available
q) Decomposition temperature	no data available
r) Viscosity	no data available
s) Explosive properties	no data available
t) Oxidizing properties	no data available



## 9.2 Other safety information

no data available

---

## 10. STABILITY AND REACTIVITY

### 10.1 Reactivity

no data available

### 10.2 Chemical stability

Stable under recommended storage conditions.

### 10.3 Possibility of hazardous reactions

no data available

### 10.4 Conditions to avoid

no data available

### 10.5 Incompatible materials

Strong oxidizing agents

### 10.6 Hazardous decomposition products

Other decomposition products - no data available

In the event of fire: see section 5

---

## 11. TOXICOLOGICAL INFORMATION

### 11.1 Information on toxicological effects

#### Acute toxicity

no data available

Inhalation: no data available

Dermal: no data available

no data available

#### Skin corrosion/irritation

no data available

#### Serious eye damage/eye irritation

no data available

#### Respiratory or skin sensitisation

no data available

#### Germ cell mutagenicity

no data available

#### Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

#### Reproductive toxicity

no data available

no data available

#### Specific target organ toxicity - single exposure

no data available

**Specific target organ toxicity - repeated exposure**

no data available

**Aspiration hazard**

no data available

**Additional Information**

RTECS: Not available

---

**12. ECOLOGICAL INFORMATION****12.1 Toxicity**

no data available

**12.2 Persistence and degradability**

no data available

**12.3 Bioaccumulative potential**

no data available

**12.4 Mobility in soil**

no data available

**12.5 Results of PBT and vPvB assessment**

PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

**12.6 Other adverse effects**

no data available

---

**13. DISPOSAL CONSIDERATIONS****13.1 Waste treatment methods****Product**

Offer surplus and non-recyclable solutions to a licensed disposal company.

**Contaminated packaging**

Dispose of as unused product.

---

**14. TRANSPORT INFORMATION****DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

---

**15. REGULATORY INFORMATION**

REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.

**SARA 302 Components**

SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**

SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**

No SARA Hazards

**Massachusetts Right To Know Components**

No components are subject to the Massachusetts Right to Know Act.

**Pennsylvania Right To Know Components**

	CAS-No.	Revision Date
EX-CELL® ACF CHO Medium	-	

**New Jersey Right To Know Components**

	CAS-No.	Revision Date
EX-CELL® ACF CHO Medium	-	

**California Prop. 65 Components**

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

---

**16. OTHER INFORMATION**

**HMIS Rating**

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

**NFPA Rating**

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

**Further information**

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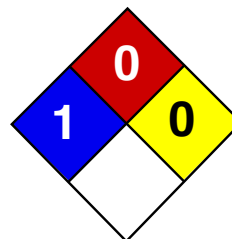
**Preparation Information**

Sigma-Aldrich Corporation  
Product Safety – Americas Region  
1-800-521-8956

Version: 4.9

Revision Date: 04/05/2014

Print Date: 04/10/2014



Health	1
Fire	0
Reactivity	0
Personal Protection	E

## Material Safety Data Sheet

### Sodium chloride MSDS

#### Section 1: Chemical Product and Company Identification

**Product Name:** Sodium chloride

**Catalog Codes:** SLS3262, SLS1045, SLS3889, SLS1669, SLS3091

**CAS#:** 7647-14-5

**RTECS:** VZ4725000

**TSCA:** TSCA 8(b) inventory: Sodium chloride

**CI#:** Not applicable.

**Synonym:** Salt; Sea Salt

**Chemical Name:** Sodium chloride

**Chemical Formula:** NaCl

#### Contact Information:

**Sciencelab.com, Inc.**

14025 Smith Rd.

Houston, Texas 77396

US Sales: **1-800-901-7247**

International Sales: **1-281-441-4400**

Order Online: [ScienceLab.com](http://ScienceLab.com)

**CHEMTREC (24HR Emergency Telephone), call:**

1-800-424-9300

**International CHEMTREC, call:** 1-703-527-3887

**For non-emergency assistance, call:** 1-281-441-4400

#### Section 2: Composition and Information on Ingredients

##### Composition:

Name	CAS #	% by Weight
Sodium chloride	7647-14-5	100

**Toxicological Data on Ingredients:** Sodium chloride: ORAL (LD50): Acute: 3000 mg/kg [Rat]. 4000 mg/kg [Mouse]. DERMAL (LD50): Acute: >10000 mg/kg [Rabbit]. DUST (LC50): Acute: >42000 mg/m 1 hours [Rat].

#### Section 3: Hazards Identification

**Potential Acute Health Effects:** Slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

##### Potential Chronic Health Effects:

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated or prolonged exposure is not known to aggravate medical condition.

#### Section 4: First Aid Measures

**Eye Contact:**

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.

**Skin Contact:**

Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used.

**Serious Skin Contact:** Not available.

**Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if symptoms appear.

**Serious Inhalation:** Not available.

**Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

**Serious Ingestion:** Not available.

## Section 5: Fire and Explosion Data

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** Not applicable.

**Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:** When heated to decomposition it emits toxic fumes.

**Special Remarks on Explosion Hazards:**

Electrolysis of sodium chloride in presence of nitrogenous compounds to produce chlorine may lead to formation of explosive nitrogen trichloride. Potentially explosive reaction with dichloromaleic anhydride + urea.

## Section 6: Accidental Release Measures

**Small Spill:**

Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.

**Large Spill:**

Use a shovel to put the material into a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

## Section 7: Handling and Storage

**Precautions:**

Keep locked up.. Do not ingest. Do not breathe dust. Avoid contact with eyes. Wear suitable protective clothing. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as oxidizing agents, acids.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area. Hygroscopic

## Section 8: Exposure Controls/Personal Protection

### Engineering Controls:

Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

### Personal Protection:

Splash goggles. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

### Personal Protection in Case of a Large Spill:

Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:** Not available.

## Section 9: Physical and Chemical Properties

**Physical state and appearance:** Solid. (Solid crystalline powder.)

**Odor:** Slight.

**Taste:** Saline.

**Molecular Weight:** 58.44 g/mole

**Color:** White.

**pH (1% soln/water):** 7 [Neutral.]

**Boiling Point:** 1413°C (2575.4°F)

**Melting Point:** 801°C (1473.8°F)

**Critical Temperature:** Not available.

**Specific Gravity:** 2.165 (Water = 1)

**Vapor Pressure:** Not applicable.

**Vapor Density:** Not available.

**Volatility:** Not available.

**Odor Threshold:** Not available.

**Water/Oil Dist. Coeff.:** Not available.

**Ionicity (in Water):** Not available.

**Dispersion Properties:** See solubility in water.

### Solubility:

Easily soluble in cold water, hot water. Soluble in glycerol, and ammonia. Very slightly soluble in alcohol. Insoluble in Hydrochloric Acid.

## Section 10: Stability and Reactivity Data

**Stability:** The product is stable.

**Instability Temperature:** Not available.

**Conditions of Instability:** Incompatible materials, high temperatures.

**Incompatibility with various substances:** Reactive with oxidizing agents, metals, acids.

**Corrosivity:** Not considered to be corrosive for metals and glass.

**Special Remarks on Reactivity:**

Hygroscopic. Reacts with most nonnoble metals such as iron or steel, building materials (such as cement) Sodium chloride is rapidly attacked by bromine trifluoride. Violent reaction with lithium.

**Special Remarks on Corrosivity:** Not available.

**Polymerization:** Will not occur.

## Section 11: Toxicological Information

**Routes of Entry:** Inhalation. Ingestion.

**Toxicity to Animals:**

WARNING: THE LC50 VALUES HEREUNDER ARE ESTIMATED ON THE BASIS OF A 4-HOUR EXPOSURE. Acute oral toxicity (LD50): 3000 mg/kg [Rat.]. Acute dermal toxicity (LD50): >10000 mg/kg [Rabbit]. Acute toxicity of the dust (LC50): >42000 mg/m<sup>3</sup> 1 hours [Rat].

**Chronic Effects on Humans:** MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast.

**Other Toxic Effects on Humans:** Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation.

**Special Remarks on Toxicity to Animals:** Lowest Published Lethal Dose (LDL) [Man] - Route: Oral; Dose: 1000 mg/kg

**Special Remarks on Chronic Effects on Humans:**

Causes adverse reproductive effects in humans (fetotoxicity, abortion, ) by intraplacental route. High intake of sodium chloride, whether from occupational exposure or in the diet, may increase risk of TOXEMIA OF PREGNANCY in susceptible women (Bishop, 1978). Hypertonic sodium chloride solutions have been used to induce abortion in late pregnancy by direct infusion into the uterus (Brown et al, 1972), but this route of administration is not relevant to occupational exposures. May cause adverse reproductive effects and birth defects in animals, particularly rats and mice (fetotoxicity, abortion, musculoskeletal abnormalities, and maternal effects (effects on ovaries, fallopian tubes) by oral, intraperitoneal, intraplacental, intrauterine, parenteral, and subcutaneous routes. While sodium chloride has been used as a negative control in some reproductive studies, it has also been used as an example that almost any chemical can cause birth defects in experimental animals if studied under the right conditions (Nishimura & Miyamoto, 1969). In experimental animals, sodium chloride has caused delayed effects on newborns, has been fetotoxic, and has caused birth defects and abortions in rats and mice (RTECS, 1997). May affect genetic material (mutagenic)

**Special Remarks on other Toxic Effects on Humans:**

Acute Potential Health Effects: Skin: May cause skin irritation. Eyes: Causes eye irritation. Ingestion: Ingestion of large quantities can irritate the stomach (as in overuse of salt tablets) with nausea and vomiting. May affect behavior (muscle spasticity/contraction, somnolence), sense organs, metabolism, and cardiovascular system. Continued exposure may produce dehydration, internal organ congestion, and coma. Inhalation: Material is irritating to mucous membranes and upper respiratory tract.

## Section 12: Ecological Information

**Ecotoxicity:** Not available.

**BOD5 and COD:** Not available.

**Products of Biodegradation:**

Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

**Toxicity of the Products of Biodegradation:** The product itself and its products of degradation are not toxic.

**Special Remarks on the Products of Biodegradation:** Not available.

### Section 13: Disposal Considerations

**Waste Disposal:**

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

### Section 14: Transport Information

**DOT Classification:** Not a DOT controlled material (United States).

**Identification:** Not applicable.

**Special Provisions for Transport:** Not applicable.

### Section 15: Other Regulatory Information

**Federal and State Regulations:** TSCA 8(b) inventory: Sodium chloride

**Other Regulations:** EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

**Other Classifications:**

**WHMIS (Canada):** Not controlled under WHMIS (Canada).

**DSCL (EEC):**

R40- Possible risks of irreversible effects. S24/25- Avoid contact with skin and eyes.

**HMIS (U.S.A.):**

**Health Hazard:** 1

**Fire Hazard:** 0

**Reactivity:** 0

**Personal Protection:** E

**National Fire Protection Association (U.S.A.):**

**Health:** 1

**Flammability:** 0

**Reactivity:** 0

**Specific hazard:**

**Protective Equipment:**

Gloves. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Splash goggles.

### Section 16: Other Information

**References:**

-Hawley, G.G.. The Condensed Chemical Dictionary, 11e ed., New York N.Y., Van Nostrand Reinold, 1987. -SAX, N.I. Dangerous Properties of Industrial Materials. Toronto, Van Nostrand Reinold, 6e ed. 1984. -The Sigma-Aldrich Library of Chemical Safety Data, Edition II.

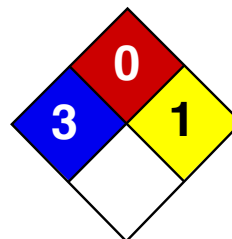
**Other Special Considerations:** Not available.

**Created:** 10/11/2005 12:33 PM



**Last Updated:** 05/21/2013 12:00 PM

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Health	3
Fire	0
Reactivity	2
Personal Protection	J

## Material Safety Data Sheet

### Sodium hydroxide, Pellets, Reagent ACS MSDS

#### Section 1: Chemical Product and Company Identification

**Product Name:** Sodium hydroxide, Pellets, Reagent ACS

**Catalog Codes:** SLS4090

**CAS#:** 1310-73-2

**RTECS:** WB4900000

**TSCA:** TSCA 8(b) inventory: Sodium hydroxide

**CI#:** Not available.

**Synonym:** Caustic Soda

**Chemical Name:** Sodium Hydroxide

**Chemical Formula:** NaOH

**Contact Information:**

**Sciencelab.com, Inc.**

14025 Smith Rd.

Houston, Texas 77396

US Sales: **1-800-901-7247**

International Sales: **1-281-441-4400**

Order Online: [ScienceLab.com](http://ScienceLab.com)

**CHEMTREC (24HR Emergency Telephone), call:**

1-800-424-9300

**International CHEMTREC, call:** 1-703-527-3887

**For non-emergency assistance, call:** 1-281-441-4400

#### Section 2: Composition and Information on Ingredients

**Composition:**

Name	CAS #	% by Weight
Sodium hydroxide	1310-73-2	100

**Toxicological Data on Ingredients:** Sodium hydroxide LD50: Not available. LC50: Not available.

#### Section 3: Hazards Identification

**Potential Acute Health Effects:**

Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion, of inhalation. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

**Potential Chronic Health Effects:**

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. The substance is toxic to lungs. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage.

## Section 4: First Aid Measures

### Eye Contact:

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

### Skin Contact:

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

### Serious Skin Contact:

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

### Inhalation:

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

### Serious Inhalation:

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. **WARNING:** It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

### Ingestion:

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

**Serious Ingestion:** Not available.

## Section 5: Fire and Explosion Data

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** of metals

### Explosion Hazards in Presence of Various Substances:

Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available. Slightly explosive in presence of heat.

**Fire Fighting Media and Instructions:** Not applicable.

### Special Remarks on Fire Hazards:

sodium hydroxide + zinc metal dust causes ignition of the latter. Under proper conditions of temperature, pressure and state of division, it can ignite or react violently with acetaldehyde, allyl alcohol, allyl chloride, benzene-1,4-diol, chlorine trifluoride, 1,2 dichlorethylene, nitroethane, nitromethane, nitroparaffins, nitropropane, cinnamaldehyde, 2,2-dichloro-3,3-dimethylbutane. Sodium hydroxide in contact with water may generate enough heat to ignite adjacent combustible materials. Phosphorous boiled with NaOH yields mixed phosphines which may ignite spontaneously in air. sodium hydroxide and cinnamaldehyde + heat may cause ignition. Reaction with certain metals releases flammable and explosive hydrogen gas.

### Special Remarks on Explosion Hazards:

Sodium hydroxide reacts to form explosive products with ammonia + silver nitrate. Benzene extract of allyl benzenesulfonate prepared from allyl alcohol, and benzene sulfonyl chloride in presence of aqueous sodium hydroxide, under vacuum distillation, residue darkened and exploded. Sodium Hydroxide + impure tetrahydrofuran, which can contain peroxides, can

cause serious explosions. Dry mixtures of sodium hydroxide and sodium tetrahydroborate liberate hydrogen explosively at 230-270 deg. C. Sodium Hydroxide reacts with sodium salt of trichlorophenol + methyl alcohol + trichlorobenzene + heat to cause an explosion.

## Section 6: Accidental Release Measures

### Small Spill:

Use appropriate tools to put the spilled solid in a convenient waste disposal container. If necessary: Neutralize the residue with a dilute solution of acetic acid.

### Large Spill:

Corrosive solid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of acetic acid. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

## Section 7: Handling and Storage

### Precautions:

Keep container dry. Do not breathe dust. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If you feel unwell, seek medical attention and show the label when possible. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, reducing agents, metals, acids, alkalis, moisture.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 23°C (73.4°F).

## Section 8: Exposure Controls/Personal Protection

### Engineering Controls:

Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

### Personal Protection:

Splash goggles. Synthetic apron. Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

### Personal Protection in Case of a Large Spill:

Splash goggles. Full suit. Vapor and dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

### Exposure Limits:

CEIL: 2 from ACGIH (TLV) [United States] [1995] Consult local authorities for acceptable exposure limits.

## Section 9: Physical and Chemical Properties

**Physical state and appearance:** Solid.

**Odor:** Odorless.

**Taste:** Not available.

**Molecular Weight:** 40 g/mole

**Color:** White.

**pH (1% soln/water):** 13.5 [Basic.]

**Boiling Point:** 1388°C (2530.4°F)  
**Melting Point:** 323°C (613.4°F)  
**Critical Temperature:** Not available.  
**Specific Gravity:** 2.13 (Water = 1)  
**Vapor Pressure:** Not applicable.  
**Vapor Density:** Not available.  
**Volatility:** Not available.  
**Odor Threshold:** Not available.  
**Water/Oil Dist. Coeff.:** Not available.  
**Ionicity (in Water):** Not available.  
**Dispersion Properties:** See solubility in water.  
**Solubility:** Easily soluble in cold water.

## Section 10: Stability and Reactivity Data

**Stability:** The product is stable.  
**Instability Temperature:** Not available.  
**Conditions of Instability:** Not available.  
**Incompatibility with various substances:**  
Highly reactive with metals. Reactive with oxidizing agents, reducing agents, acids, alkalis, moisture.  
**Corrosivity:** Not available.  
**Special Remarks on Reactivity:**  
Hygroscopic. Much heat is evolved when solid material is dissolved in water. Therefore cold water and caution must be used for this process. Sodium hydroxide solution and octanol + diborane during a work-up of a reaction mixture of oxime and diborane in tetrahydrofuran is very exothermic, a mild explosion being noted on one occasion. Reactive with water, acids, acid chlorides, strong bases, strong oxidizing agents, strong reducing agents, flammable liquids, organic halogens, metals (i.e aluminum, tin, zinc), nitromethane, glacial acetic acid, acetic anhydride, acrolein, chlorohydrin, chlorosulfonic acid, ethylene cyanohydrin, glyoxal, hydrochloric acid, sulfuric acid, hydrosulfuric acid, nitric acid, oleum, propiolactone, acrylonitrile, phosphorus pentoxide, chloroethanol, chloroform-methanol, tetrahydroborate, cyanogen azide, 1,2,4,5 tetrachlorobenzene, cinnamaldehyde. Reacts with formaldehyde hydroxide to yield formic acid, and hydrogen.  
**Special Remarks on Corrosivity:** Very caustic to aluminum and other metals in presence of moisture.  
**Polymerization:** Will not occur.

## Section 11: Toxicological Information

**Routes of Entry:** Absorbed through skin. Dermal contact. Eye contact. Inhalation. Ingestion.  
**Toxicity to Animals:**  
LD50: Not available. LC50: Not available.  
**Chronic Effects on Humans:** Causes damage to the following organs: lungs.  
**Other Toxic Effects on Humans:**  
Extremely hazardous in case of inhalation (lung corrosive). Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (corrosive), of ingestion, .  
**Special Remarks on Toxicity to Animals:**

Lowest Published Lethal Dose: LDL [Rabbit] - Route: Oral; Dose: 500 mg/kg

**Special Remarks on Chronic Effects on Humans:** May affect genetic material (mutagenic). Investigation as a mutagen (cytogenetic analysis), but no data available.

**Special Remarks on other Toxic Effects on Humans:**

Acute Potential Health Effects: Skin: May be harmful if absorbed through skin. Causes severe skin irritation and burns. May cause deep penetrating ulcers of the skin. Eyes: Causes severe eye irritation and burns. May cause chemical conjunctivitis and corneal damage. Inhalation: Harmful if inhaled. Causes severe irritation of the respiratory tract and mucous membranes with coughing, burns, breathing difficulty, and possible coma. Irritation may lead the chemical pneumonitis and pulmonary edema. Causes chemical burns to the respiratory tract and mucous membranes. Ingestion: May be fatal if swallowed. May cause severe and permanent damage to the digestive tract. Causes severe gastrointestinal tract irritation and burns. May cause perforation of the digestive tract. Causes severe pain, nausea, vomiting, diarrhea, and shock. May cause corrosion and permanent destruction of the esophagus and digestive tract.

## Section 12: Ecological Information

**Ecotoxicity:** Not available.

**BOD5 and COD:** Not available.

**Products of Biodegradation:**

Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

**Toxicity of the Products of Biodegradation:** The product itself and its products of degradation are not toxic.

**Special Remarks on the Products of Biodegradation:** Not available.

## Section 13: Disposal Considerations

**Waste Disposal:**

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

## Section 14: Transport Information

**DOT Classification:** Class 8: Corrosive material

**Identification:** : Sodium hydroxide, solid UNNA: 1823 PG: II

**Special Provisions for Transport:** Not available.

## Section 15: Other Regulatory Information

**Federal and State Regulations:**

Illinois toxic substances disclosure to employee act: Sodium hydroxide Illinois chemical safety act: Sodium hydroxide New York release reporting list: Sodium hydroxide Rhode Island RTK hazardous substances: Sodium hydroxide Pennsylvania RTK: Sodium hydroxide Minnesota: Sodium hydroxide Massachusetts RTK: Sodium hydroxide New Jersey: Sodium hydroxide Louisiana spill reporting: Sodium hydroxide California Director's List of Hazardous Substances: Sodium hydroxide TSCA 8(b) inventory: Sodium hydroxide CERCLA: Hazardous substances.: Sodium hydroxide: 1000 lbs. (453.6 kg)

**Other Regulations:**

OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200). EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

**Other Classifications:**

**WHMIS (Canada):** CLASS E: Corrosive solid.

**DSCL (EEC):**

**HMIS (U.S.A.):**

**Health Hazard:** 3

**Fire Hazard:** 0

**Reactivity:** 2

**Personal Protection:** j

**National Fire Protection Association (U.S.A.):**

**Health:** 3

**Flammability:** 0

**Reactivity:** 1

**Specific hazard:**

**Protective Equipment:**

Gloves. Synthetic apron. Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate. Splash goggles.

## Section 16: Other Information

**References:** Not available.

**Other Special Considerations:** Not available.

**Created:** 10/09/2005 06:32 PM

**Last Updated:** 05/21/2013 12:00 PM

*The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall ScienceLab.com be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if ScienceLab.com has been advised of the possibility of such damages.*

# MATERIAL SAFETY DATA SHEET

## SECTION 1 - IDENTIFICATION OF THE MATERIAL AND SUPPLIER

**Material Name:** Phosphate buffered saline, pH 7.4  
**Catalogue Number:** APBS-1L  
**Other Names:** Sorenson's Saline; Phosphate Buffered Saline.  
**Recommended Use:** Used as a buffer or cell wash dilution in microscopy in hospital and pathology laboratories.

**Supplier Name:** ProSciTech  
**Street Address:** 1/11 Carlton Street, Kirwan, Qld. 4817 Australia  
**Telephone Number:** (07) 4773 9444 - 8:30am – 5:00pm, Monday to Friday (excluding Public Holidays)  
**Emergency Contact:** (07) 4773 9444 - 8:30am – 5:00pm, Monday to Friday (excluding Public Holidays)

## SECTION 2 - HAZARDS IDENTIFICATION

**Hazard Classification:**

Not classified as hazardous according to criteria for Classifying Hazardous Substances [NOHSC:1008].

**Hazardous and/or Dangerous Nature:**

NON-HAZARDOUS SUBSTANCE. NON-DANGEROUS GOODS.

**Risk Phrases:**

None allocated.

**Safety Phrases:**

S24/25: Avoid contact with skin and eyes.

*Refer to Section 15 for Poisons Schedule.*

## SECTION 3 - COMPOSITION /INFORMATION ON INGREDIENTS

**Mixture Substance:**

<i>Ingredients</i>	<i>Cas Number(s)</i>	<i>Proportion (%)</i>
SODIUM CHLORIDE	7647-14-5	0.8
POTASSIUM DIHYDROGEN PHOSPHATE	7778-77-0	0.02
DISODIUM HYDROGEN ORTHOPHOSPHATE	7558-79-4	0.1
POTASSIUM CHLORIDE	7447-40-7	0.02
WATER	7732-18-5	99

## SECTION 4 - FIRST AID MEASURES

**Ingestion:** If swallowed, DO NOT induce vomiting. If victim is conscious give water. If sickness persists transport to hospital or doctor.

**Inhalation:** Move victim to fresh air. Apply resuscitation if victim is not breathing - If trained personnel available administer oxygen if breathing is difficult.

**Eye Contact:** If material is splashed into eyes, immediately, flush with plenty of water for 15 minutes, ensuring eye lids are held open. If irritation persists transport to hospital or doctor.

**Skin Contact:** If material is splashed onto the skin, remove any contaminated clothing and wash skin thoroughly with water and soap if available. If irritation persists transport to hospital or doctor.

**First Aid Facilities:** Eyebath/eyewash, Safety shower & general washroom facilities.

**Medical Attention & Special Treatment:**  
Treat symptomatically.

**Additional Information:**  
Not available.

## SECTION 5 - FIRE FIGHTING MEASURES

**Suitable Extinguishing Media:**

Use extinguishing media suitable for surrounding fire situation. This material is not a flammable or combustible liquid.

**Hazards from Combustion Products:**

Decomposes on heating emitting oxides of carbon.

**Precautions for Fire Fighters:**

Fire fighters to wear Self-contained breathing apparatus (SCBA) in confined spaces, in oxygen deficient atmospheres or if exposed to products of decomposition. Full protective clothing is also recommended. If safe to do so, move undamaged containers from fire area.





# MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

**Part Number(s):**  
54-111-04, 54-111-05

**Product Name:** Sterilizable Cheek Retractor

**Release Date:** April 10, 2007

## 1. Product and Company Identification

<b>Trade Name &amp; Synonyms</b> Sterilizable cheek retractor	<b>MSDS Code Number</b> CKE 12, 2S
<b>Chemical Name</b> Polyethersulfone-polymer. Chemical family: Polyethersulfone.	<b>Manufacture / Distributor</b> GAC International Inc.
<b>C.A.S. Number</b> 25154-01-2	<b>Address</b> 355 Knickerbocker Ave. Bohemia, NY USA 11716
<b>Grades or Minor Variant Identities</b> NA	<b>Information Telephone Number</b> 1-631-419-1700
<b>Product Use (for Canada)</b> NA	<b>Emergency Telephone Number</b> 1-631-419-1700

## 2. Composition of Ingredients

<b>Hazardous Components</b>	<b>C.A.S. Number</b>	<b>Exposure Limits</b>	<b>%</b>
Polyethersulfone	25154-01-2	N/A	NA

## 3. Hazard Identification

### Emergency Overview

This product has non-hazardous ingredient as defined under the criteria of the federal OSHA hazard communication standard 29 CFR 1910.1200.

<b>Routes of Exposure</b>	<b>Signs and Symptoms</b>	<b>Single, Repeated, or Lifetime Exposure</b>	<b>Severity (Mild, Moderate, Severe)</b>	<b>Acute and Chronic Health Effect(s)</b>	<b>Target Organ(s)</b>
Eye	NA	NA	NA	Acute: Mechanical irritation. Chronic: None	NA
Skin	NA	NA	NA	NA	NA
Inhalation	NA	NA	NA	Acute: No significant health hazard. Chronic: None.	NA
Ingestion	NA	NA	NA	NA	NA
Other	NA	NA	NA	NA	NA

### Medical Conditions Aggravated by Exposure

None. Human effects and symptoms of over exposure: None

### Carcinogenicity (IARC, NTP)

None

### Potential Environmental Effects

Potential health effects: None. Exposure limits: None.

## 4. First Aid Measures

<b>Routes of Exposure</b>	<b>First Aid Instructions</b>	<b>Immediate Medical Attention</b>	<b>Delayed Effects</b>
Eye	N/A	NA	NA
Skin	N/A	NA	NA
Inhalation	N/A	NA	NA
Ingestion	N/A	NA	NA
Other	NA	NA	NA

### Note to Physicians (Treatment, Testing, and Monitoring)

NA



# MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

**Part Number(s):**  
54-111-04, 54-111-05

**Product Name:** Sterilizable Cheek Retractor

**Release Date:** April 10, 2007

## 5. Fire and Explosion Data

<b>Flashpoint &amp; Method:</b> °C / °F Non-flammable	<b>Flammable (Explosive) Limits in Air</b> LEL: N/A                      UEL: N/A		<b>Autoignition Temperature</b> NA	<b>Other</b> NA
<b>Flame Propagation or Burning</b> NA	<b>Properties Contributing to Fire Intensity</b> NA		<b>Flammability Classification</b> Health- 0, Flammability-0 , Reactivity-0	
<b>Extinguishing Media</b> N/A			<b>Extinguishing Media to Avoid</b> NA	
<b>Protection and Procedures for Firefighters</b> None				
<b>Unusual Fire and Explosion Hazards</b> NA				

## 6. Accidental Release Measures

<b>Containment Techniques</b> NA
<b>Spill/Leak Clean-Up Procedures and Equipment</b> Remove by mechanical means.
<b>Evacuation Procedures</b> NA
<b>Special Instructions</b> NA
<b>Reporting Requirements</b> NA

## 7. Handling and Storage

<b>Handling Practices and Warnings</b> None
<b>Storage Practices and Warnings</b> (°C / °F). Storage temperature: Ambient. <b>Shelf life:</b> 3 years.

## 8. Exposure Control/Personal Protection

<b>Ventilation</b> None		<b>Other Engineering Controls</b> NA
<b>Routes of Entry:</b>	<b>Personal Protective Equipment (PPE) for Normal Use:</b>	<b>PPE for Emergencies:</b>
<b>Eye/Face</b>	None	NA
<b>Skin</b>	None	NA
<b>Inhalation</b>	None	NA
<b>General Hygiene Considerations and Work Practices</b> NA		
<b>Protective Measures During Repair and Maintenance of Contaminated Equipment</b> NA		
<b>Other Protective Measures and Equipment</b> None		



# MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

Part Number(s):  
54-111-04, 54-111-05

Product Name: Sterilizable Cheek Retractor

Release Date: April 10, 2007

## 9. Physical and Chemical Characteristics

<b>Appearance</b> Color: Various F.D.A allowable colors.		<b>Odor</b> Odorless
<b>Normal Physical State:</b> <input type="checkbox"/> Liquid <input type="checkbox"/> Gas <input checked="" type="checkbox"/> Solid <input type="checkbox"/> _____ (Other)		<b>Boiling Point</b> °C / °F    N/A <b>Melting Point</b> °C / °F    N/A <b>Freezing Point</b> °C / °F    N/A
<b>Specific Gravity or Density (H<sub>2</sub>O=1)</b> 1.37 g/cm <sup>3</sup>	<b>Solubility in Water</b> Insoluble	<b>pH</b> N/D
<b>Vapor Pressure (mm Hg @ 20°C)</b> N/A	<b>Vapor Density (AIR= 1)</b> NA	<b>Evaporation Rate (Butyl Acetate = 1)</b> NA
<b>Other</b> Bulk density: Approximately 10.01 LBS /CM <sup>3</sup> . % Volatile by volume: N/A		

## 10. Stability and Reactivity Data

<b>Incompatibility (Materials to Avoid)</b> None known.	
<b>Hazardous Products Produced During Decomposition</b> By fire; carbon monoxide, carbon dioxide.	
<b>Hazardous Polymerization?</b> <input type="checkbox"/> May Occur <input checked="" type="checkbox"/> May Not Occur	<b>Conditions to Avoid</b> °C / °F    NA
<b>Stability?</b> <input checked="" type="checkbox"/> Stable <input type="checkbox"/> Unstable	<b>Conditions to Avoid</b> °C / °F    None known.

## 11. Toxicological Information

<b>Toxicity Data, Epidemiology Studies, Carcinogenicity, Neurological Effects, Genetic or Reproductive Effects, or Structure Activity Data</b> No component of this product present at levels greater than 0.1 % is identified as a carcinogen by the U.S. National toxicology program, the U.S. occupational safety and health act, or the international agency on research on cancer (IARC).
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## 12. Ecological Information

<b>Toxicity, Environmental Fate, Physical/Chemical Data, or Other Data Supporting Environmental Hazard Statements</b> N/A
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## 13. Disposal Considerations

<b>Regulations</b> Waste disposal method: In accordance to federal, state or local regulations.
<b>Properties (Physical/Chemical) Affecting Disposal</b> NA



# MATERIAL SAFETY DATA SHEET

Form WI04-11A Rev. 1

**Part Number(s):**  
54-111-04, 54-111-05

**Product Name:** Sterilizable Cheek Retractor

**Release Date:** April 10, 2007

## 14. Transport Information

<b>Regulated for shipping?</b> <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<b>Proper Shipping Name</b> Sterilizable cheek retractor	<b>Packing Group</b> NA
<b>Do changes in quantity, packaging, or shipment method change product classification?</b> <input type="checkbox"/> Yes <input type="checkbox"/> No NA	<b>Hazard Class</b> Not regulated	<b>Identification Number</b> NA
<b>Other</b> Freight class bulk: Not regulated. Freight class package: Not regulated. Product label: Not regulated. Hazard class division number: Not regulated		

## 15. Regulatory Information

<b>Federal Regulations</b> Sara title III: This product is not reportable under section 302.4 of SARA and 40 CFR part 355. Hazardous substances: None. Section 311/312 Hazard categories: None. Section 313 Toxic chemicals: None RERA Status: Non-hazardous.
<b>International Regulations</b> NA
<b>Other</b> NA

## 16. Other Information

<b>Supplier Number:</b> 31/ 54	<b>Supplier Release:</b> NA
N/A = not applicable. NA = not available, N/E = not established. N/D = not determined.	

## MATERIAL SAFETY DATA SHEET: Recombinant Protein A (Revision 1)

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### SECTION 1 – PRODUCT IDENTIFICATION

Supplier: RepliGen Corporation  
41 Seyon Street, Building #1, Suite 100  
Waltham, MA 02453  
Phone: (781) 250-0111; Fax: (781) 250-0115

Product Name: Recombinant Protein A  
Synonyms: rPA50, srPA50, rSPA, rProteinA, rProtein A, Protein A  
Catalog No(s): 10-1001, 10-1501, 10-2001

---

### SECTION 2 – COMPOSITION / INFORMATION ON INGREDIENTS

Purified Recombinant Protein A, derived from genetically modified *Escherichia coli*. Product is provided frozen in an aqueous buffer.

---

### SECTION 3 – HAZARDS IDENTIFICATION

Emergency Overview: No specific hazards identified

HMIS: Health Hazard: 0 (No significant risk to health.)  
Flammability: 0 (Will not burn)  
Reactivity: 0 (Stable)

NFPA: Health Hazard: 0 (Poses no health hazard,)  
Fire: 0 (Will not burn)  
Reactivity: 0 (Stable, not reactive with water)

Potential Health Effects: No health effects have been identified.  
May be harmful if inhaled, swallowed, or absorbed through skin.  
May cause eye irritation.

---

### SECTION 4 – FIRST AID MEASURES

If swallowed: Induce vomiting. Get medical attention  
In case of eye contact: Flush eyes with clean water for at least 15 minutes  
Skin contact: Flush skin with water  
If inhaled: Move to fresh air. Get medical attention

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### SECTION 5 – FIRE FIGHTING MEASURES

Non Flammable: No specific fire hazard  
Flash point: N/A  
Ignition point: N/A  
Fire Extinguishing media: Use any suitable media as for the surrounding fire

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**Recombinant Protein A (rPA50, srPA50, rSPA, rProteinA, rProtein A, Protein A)**

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**SECTION 6 – STEPS TO BE TAKEN IN THE EVENT OF A SPILL OR DISCHARGE:**

<u>Personal Protection</u>	Wear lab coat, gloves and eye protection. Treat with procedures appropriate for biological materials. Soak up spill with absorbent material and collect in a closed container suitable for incineration. Clean the affected area with disinfectant solution. Do not allow material to enter soil, waterways or drains.
<u>Disposal procedure:</u>	Dispose of in accordance with all applicable federal, state, and local environmental regulations.

---

**SECTION 7 – HANDLING AND STORAGE**

Ventilation:	Keep in a well ventilated area
Respiratory Protection:	N/A
Eye/skin Protection	Standard laboratory practices recommended.
Storage:	Keep container closed. Store frozen for optimum shelf life.
Special precautions:	N/A

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**SECTION 8 – EXPOSURE CONTROLS/PERSONAL PROTECTION**

General	Standard laboratory practices recommended. Clean any exposed skin after handling, before leaving the working area, and before eating, smoking or using the lavatory. Dispose of, or clean any contaminated clothing before re-use.
PPE	Personal protective equipment should be selected to provide adequate protection based upon the procedures being performed. Wear laboratory coat, gloves and safety glasses when handling. Respiratory protection not required

---

**SECTION 9 – PHYSICAL AND CHEMICAL PROPERTIES**

Appearance:	Frozen aqueous solution
pH	pH 5 - 8
Flash point	Will not burn
Ignition point	Will not ignite
Explosion limits	No risk of explosion
Solubility	Soluble in water

---

**SECTION 10 – STABILITY AND REACTIVITY**

Stability:	Stable
Hazardous polymerization:	Will not occur
Decomposition products:	No known hazardous decomposition products

---

**SECTION 11 – TOXICOLOGICAL INFORMATION**

Acute toxicity:	No known significant effects
Irritation:	No known significant effects. May be a skin or eye irritant.
Sensitization:	No known significant effects
Carcinogenicity	No known significant effects
Mutagenicity	No known significant effects
Teratogenicity	No known significant effects

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**Recombinant Protein A (rPA50, srPA50, rSPA, rProteinA, rProtein A, Protein A)**

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**SECTION 12 – ECOLOGICAL INFORMATION**

No known hazards

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**SECTION 13 – DISPOSAL CONSIDERATIONS**

Dispose of in accordance with all applicable federal, state, and local environmental regulations.  
Do not allow spilled material to enter soil, waterways or drains

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**SECTION 14 – TRANSPORT INFORMATION**

IATA	Not classified
DOT Road Transport	Not Regulated

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**SECTION 15 – REGULATORY INFORMATION**

OSHA/SARA/CWA/CAA: No known hazards

EU Risk and Safety Statements:

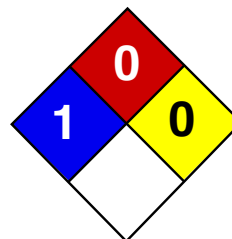
- Not classified
- 

**SECTION 16 – OTHER INFORMATION**

The material published in this Material Safety Data Sheet has been compiled from our experience and data presented in various technical publications. It is the user's responsibility to determine the suitability of this information for the adoption of necessary safety precautions.

Repligen makes no warranty or representation about the accuracy or completeness nor fitness for purpose of the information contained herein.

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Health	1
Fire	0
Reactivity	0
Personal Protection	E

## Material Safety Data Sheet

### Sodium bicarbonate MSDS

#### Section 1: Chemical Product and Company Identification

**Product Name:** Sodium bicarbonate

**Catalog Codes:** SLS3241, SLS2446, SLS3868

**CAS#:** 144-55-8

**RTECS:** VZ0950000

**TSCA:** TSCA 8(b) inventory: Sodium bicarbonate

**CI#:** Not available.

**Synonym:** Baking Soda; Bicarbonate of soda; Sodium acid carbonate; Monosodium carbonate; Sodium hydrogen carbonate; Carbonic acid monosodium salt

**Chemical Name:** Sodium Bicarbonate

**Chemical Formula:** NaHCO<sub>3</sub>

#### Contact Information:

**Sciencelab.com, Inc.**

14025 Smith Rd.

Houston, Texas 77396

US Sales: **1-800-901-7247**

International Sales: **1-281-441-4400**

Order Online: [ScienceLab.com](http://ScienceLab.com)

**CHEMTREC (24HR Emergency Telephone), call:**

1-800-424-9300

**International CHEMTREC, call:** 1-703-527-3887

**For non-emergency assistance, call:** 1-281-441-4400

#### Section 2: Composition and Information on Ingredients

##### Composition:

Name	CAS #	% by Weight
Sodium bicarbonate	144-55-8	100

**Toxicological Data on Ingredients:** Not applicable.

#### Section 3: Hazards Identification

**Potential Acute Health Effects:** Slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

##### Potential Chronic Health Effects:

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated or prolonged exposure is not known to aggravate medical condition.

#### Section 4: First Aid Measures

##### Eye Contact:

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention if irritation occurs.



**Skin Contact:**

Wash with soap and water. Cover the irritated skin with an emollient. Get medical attention if irritation develops. Cold water may be used.

**Serious Skin Contact:** Not available.

**Inhalation:**

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

**Serious Inhalation:** Not available.

**Ingestion:**

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

**Serious Ingestion:** Not available.

## Section 5: Fire and Explosion Data

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** Not applicable.

**Explosion Hazards in Presence of Various Substances:**

Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:** When heated to decomposition it emits acrid smoke and irritating fumes.

**Special Remarks on Explosion Hazards:** Not available.

## Section 6: Accidental Release Measures

**Small Spill:**

Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.

**Large Spill:**

Use a shovel to put the material into a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

## Section 7: Handling and Storage

**Precautions:**

Do not ingest. Do not breathe dust. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as acids.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area.

## Section 8: Exposure Controls/Personal Protection

**Engineering Controls:**

Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

**Personal Protection:** Safety glasses. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

**Personal Protection in Case of a Large Spill:**

Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

**Exposure Limits:** Not available.

## Section 9: Physical and Chemical Properties

**Physical state and appearance:** Solid.

**Odor:** Odorless.

**Taste:** Saline. Alkaline.

**Molecular Weight:** 84.01g/mole

**Color:** White.

**pH (1% soln/water):** Not available.

**Boiling Point:** Not available.

**Melting Point:** Not available.

**Critical Temperature:** Not available.

**Specific Gravity:** Density: 2.159 (Water = 1)

**Vapor Pressure:** Not applicable.

**Vapor Density:** Not available.

**Volatility:** Not available.

**Odor Threshold:** Not available.

**Water/Oil Dist. Coeff.:** Not available.

**Ionicity (in Water):** Not available.

**Dispersion Properties:** See solubility in water.

**Solubility:**

Soluble in cold water. Slightly soluble in alcohol. Solubility in Water: 6.4, 7.6, 8.7, 10.0, 11.3, 12.7, 14.2, 16.5, 19.1 g/100 solution at 0, 10, 20, 30, 40, 50, 60, 80, and 100 deg. C, respectively. Solubility in Water: 6.9, 8.2, 9.6, 11.1, 12.7, 14.5, 16.5, 19.7, and 23.6 g/100g water at 0, 10, 20, 30, 40, 50, 60, 80, 100 deg. C, respectively.

## Section 10: Stability and Reactivity Data

**Stability:** The product is stable.

**Instability Temperature:** Not available.

**Conditions of Instability:** Incompatible materials, Moisture. Stable in dry air, but slowly decomposes in moist air.

**Incompatibility with various substances:** Reactive with acids.

**Corrosivity:** Non-corrosive in presence of glass.

**Special Remarks on Reactivity:**

Reacts with acids to form carbon dioxide. Dangerous reaction with monoammonium phosphate or a sodium-potassium alloy.

**Special Remarks on Corrosivity:** Not available.

**Polymerization:** Will not occur.

## Section 11: Toxicological Information

**Routes of Entry:** Inhalation. Ingestion.

**Toxicity to Animals:** Acute oral toxicity (LD50): 3360 mg/kg [Mouse].

**Chronic Effects on Humans:** Not available.

**Other Toxic Effects on Humans:** Slightly hazardous in case of skin contact (irritant), of ingestion, of inhalation.

**Special Remarks on Toxicity to Animals:** Not available.

**Special Remarks on Chronic Effects on Humans:**

Sodium Bicarbonate as produced genetic effects in rats (unscheduled DNA synthesis). However, no affects have been found in humans.

**Special Remarks on other Toxic Effects on Humans:**

Acute Potential Health Effects: Skin: May cause mild skin irritation. Eyes: May cause mild eye irritation. Inhalation: May cause respiratory tract irritation. Symptoms may include coughing and sneezing. Ingestion: Symptoms of overexposure to Sodium Bicarbonate include thirst, abdominal pain, gastroenteritis, and inflammation of the digestive tract. Chronic Potential Health Effects: Skin: Repeated or prolonged skin contact may cause irritation, drying or cracking of the skin. Ingestion and Inhalation: Chronic toxicity usually occurs within 4 to 10 days following ingestion of very large amounts. Repeated or prolonged ingestion or inhalation of large amounts may cause metabolic abnormalities, and sodium retention. Metabolic abnormalities such as acidosis, hypernatremia, hyponatremia, alkalosis, hypocalcemia, or sodium retention may affect the blood, kidneys, respiration (cyanosis, apnea secondary to metabolic acidosis or pulmonary edema), and cardiovascular system (tachycardia, hypotension). Severe toxicity may also affect behavior/central nervous system/nervous system. Neurological changes may result from metabolic abnormalities. These may include fatigue, irritability, dizziness, mental confusion, paresthesia, seizures, tetany, cerebral edema. Medical Conditions Aggravated by Exposure: Persons with pre-existing skin conditions might have increased sensitivity. Predisposing conditions that contribute to a mild alkali syndrome include, renal disease, dehydration, and electrolyte imbalance, hypertension, sarcoidosis, congestive heart failure, edema, or other sodium retaining conditions.

## Section 12: Ecological Information

**Ecotoxicity:** Not available.

**BOD5 and COD:** Not available.

**Products of Biodegradation:**

Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

**Toxicity of the Products of Biodegradation:** The product itself and its products of degradation are not toxic.

**Special Remarks on the Products of Biodegradation:** Not available.

## Section 13: Disposal Considerations

**Waste Disposal:**

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

## Section 14: Transport Information

**DOT Classification:** Not a DOT controlled material (United States).

**Identification:** Not applicable.

**Special Provisions for Transport:** Not applicable.

## Section 15: Other Regulatory Information

**Federal and State Regulations:** TSCA 8(b) inventory: Sodium bicarbonate

**Other Regulations:** Not available.

**Other Classifications:**

**WHMIS (Canada):** Not controlled under WHMIS (Canada).

**DSCL (EEC):**

This product is not classified according to the EU regulations. Not applicable.

**HMIS (U.S.A.):**

**Health Hazard:** 1

**Fire Hazard:** 0

**Reactivity:** 0

**Personal Protection:** E

**National Fire Protection Association (U.S.A.):**

**Health:** 1

**Flammability:** 0

**Reactivity:** 0

**Specific hazard:**

**Protective Equipment:**

Gloves. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Safety glasses.

## Section 16: Other Information

**References:** Not available.

**Other Special Considerations:** Not available.

**Created:** 10/10/2005 08:26 PM

**Last Updated:** 05/21/2013 12:00 PM

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## MATERIAL SAFETY DATA SHEET

REF: 91/155/EEC AND AMENDMENTS WITH RESPECTIVE NATIONAL IMPLEMENTATIONS

### SODIUM CITRATE

#### 1.0 SUBSTANCE IDENTIFICATION

---

- 1.1 Commercial product name: Sodium Citrate including anhydrous, dehydrate etc
- 1.2 Chemical characterisation: Trisodium salt of 2-hydroxypropane-1,2,3, tricarboxylate
- 1.3 Formula:
- 1.4 Molecular weight: approx 258 - 294
- 1.5 CAS No: 6132-04-3
- 1.6 EINECS No.: 200-675-3
- 1.7 **FOR USE IN FOOD as a food additive**
- 1.8 Manufactured by: Archer Daniels Midland Company, 4666 Faries Parkway,  
Decatur, Illinois 62526, U.S.A.
- 1.9 Supplied by: ADM Australia Pty Ltd, PO Box 281, Suite 1003, Level 10,  
1 Newland Street, Bondi Junction, NSW 2022
- 1.10 **Australian Emergency Telephone Number: 0417285396**

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#### 2.0 COMPOSITION

- 2.1 Totally 100% sodium citrate
- 2.2 Volatile matter 6-12 % maximum after drying for 4 hours at 180°C
- 2.3 Sodium ion content typically 23 - 24%

#### 3.0 HAZARDS IDENTIFICATION

- 3.1 **Sodium citrate is not classified as a Dangerous Substance within the definitions of EC Directive 67/584**
- 3.2 Contact with eyes (e.g. dust particles) may cause irritation.

#### 4.0 FIRST AID MEASURES

- 4.1 Flush affected parts with plenty of water

#### 5.0 FIRE FIGHTING MEASURES

- 5.1 All types of fire extinguisher are suitable
- 5.2 Firefighters wear protective clothing and NIOSH approved respirator

#### 6.0 ACCIDENTAL RELEASE MEASURES

- 6.1 After spillage/leakage : Recover by vacuum, or broom and shovel. Flush area with water to remove final traces.

#### 7.0 HANDLING AND STORAGE

- 7.1 Store in tightly closed containers, away from extreme heat and humidity. Maximum 25°C and 50% relative humidity.
- 7.2 Industrial Hygiene: Good ventilation required if process creates the formation of dust.

## 8.0 EXPOSURE CONTROLS / PERSONAL PROTECTION

- |     |                         |   |
|-----|-------------------------|---|
| 8.1 | Personal precautions:   | Avoid breathing dust.<br>Avoid contact with eyes. |
| 8.2 | Respiratory protection: | Approved nuisance dust mask                       |
| 8.3 | Hand protection:        | Standard work gloves                              |
| 8.4 | Eye protection:         | Goggles or safety glasses                         |

## 9.0 PHYSICAL AND CHEMICAL PROPERTIES

- |      |   |
|------|---|
| 9.1  | Appearance: Crystals  |
| 9.2  | Colour: Colourless  |
| 9.3  | Odour: Odourless  |
| 9.4  | Molecular weight: 258 - 294   |
| 9.5  | Change in physical state: Loss of water above 180°C, with decomposition             |
| 9.6  | Specific gravity Bulk density: 1.97 920-1150 kg/m <sup>3</sup> (typical range)      |
| 9.8  | Vapour pressure: N/A - solid  |
| 9.9  | Viscosity: N/A - solid  |
| 9.10 | Solubility - in water (25°C) 40 - 60% w/w<br>- in ethanol (25°C) Insoluble          |
| 9.11 | pH (5% solution) (25°C) 7 - 9   |
| 9.12 | Flash point N/A   |
| 9.13 | Explosive properties N/A  |
| 9.14 | Flammability: Requires external heat to burn  |
| 9.12 | Thermal decomposition: Above 230°C may evolve carbon monoxide<br>and carbon dioxide |

## 10.0 STABILITY AND REACTIVITY

- |      |              |  |
|------|--------------|--|
| 10.1 | Shelf life : | Sodium citrate is chemically stable if stored under cool, dry conditions; 25°C maximum and 50% relative humidity. It deliquesces in moist air. Physical properties may change on storage: re-test recommended periodically based on actual storage conditions. |
| 10.4 | Reactivity:  | Sodium citrate is a neutral salt with low activity.  |

## 11.0 TOXICOLOGICAL INFORMATION

- 11.1 LD<sub>50</sub> (dog): Not available

## 12.0 ECOLOGICAL INFORMATION

- |      |               |
|------|---------------|
| 12.1 | Not Available |
| 12.2 | Not Available |

### **13.0 DISPOSAL CONSIDERATIONS**

13.1 Sodium citrate is suitable for landfill or disposal to sewer **depending upon local regulations.**

### **14.0 TRANSPORT INFORMATION**

14.1 No special considerations

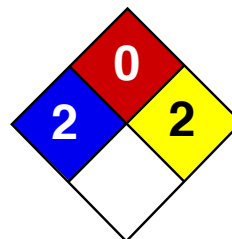
### **15.0 REGULATORY INFORMATION**

15.1 Sodium citrate is an EU permitted Food Additive (E 332). Conditions of use: Quantum Satis. The US Food and Drug Administration classifies potassium citrate as a GRAS (Generally Recognised As Safe) food ingredient.

15.2 According to the Joint Expert Committee on Food Additives of WHO/FAO potassium Citrate may be used without limitation according to Good Manufacturing Practices.

### **16.0 ADDITIONAL INFORMATION**

16.1 See Product Data Sheet.



Health	2
Fire	0
Reactivity	0
Personal Protection	C

## Material Safety Data Sheet

### Sodium phosphate tribasic MSDS

#### Section 1: Chemical Product and Company Identification

**Product Name:** Sodium phosphate tribasic

**Catalog Codes:** SLS2650, SLS4072

**CAS#:** 7601-54-9

**RTECS:** TC9490000

**TSCA:** TSCA 8(b) inventory: Sodium phosphate tribasic

**CI#:** Not available.

**Synonym:** Trisodium Phosphate Anhydrous; Phosphoric Acid, Trisodium Salt; Trisodium Orthophosphate

**Chemical Name:** Sodium Phosphate Tribasic

**Chemical Formula:** Na<sub>3</sub>PO<sub>4</sub>

#### Contact Information:

**Sciencelab.com, Inc.**

14025 Smith Rd.

Houston, Texas 77396

US Sales: **1-800-901-7247**

International Sales: **1-281-441-4400**

Order Online: [ScienceLab.com](http://ScienceLab.com)

**CHEMTREC (24HR Emergency Telephone), call:**

1-800-424-9300

**International CHEMTREC, call:** 1-703-527-3887

**For non-emergency assistance, call:** 1-281-441-4400

#### Section 2: Composition and Information on Ingredients

##### Composition:

Name	CAS #	% by Weight
Sodium phosphate tribasic	7601-54-9	100

**Toxicological Data on Ingredients:** Sodium phosphate tribasic: ORAL (LD50): Acute: 4150 mg/kg [Rat [information from other supplier]]. DERMAL (LD50): Acute: >7940 mg/kg [Rabbit [information from other supplier]]. >300 mg/kg [Rabbit [Registry of Toxic Effects of Chemical Substances database]].

#### Section 3: Hazards Identification

##### Potential Acute Health Effects:

Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator). Corrosive to eyes and skin. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death.

##### Potential Chronic Health Effects:

CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage.



## Section 4: First Aid Measures

### Eye Contact:

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

### Skin Contact:

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

### Serious Skin Contact:

Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

### Inhalation:

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

**Serious Inhalation:** Not available.

### Ingestion:

Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

**Serious Ingestion:** Not available.

## Section 5: Fire and Explosion Data

**Flammability of the Product:** Non-flammable.

**Auto-Ignition Temperature:** Not applicable.

**Flash Points:** Not applicable.

**Flammable Limits:** Not applicable.

**Products of Combustion:** Not available.

**Fire Hazards in Presence of Various Substances:** Not applicable.

### Explosion Hazards in Presence of Various Substances:

Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available. Slightly explosive in presence of heat.

**Fire Fighting Media and Instructions:** Not applicable.

**Special Remarks on Fire Hazards:** Not available.

**Special Remarks on Explosion Hazards:** Containers may explode when heated

## Section 6: Accidental Release Measures

### Small Spill:

Use appropriate tools to put the spilled solid in a convenient waste disposal container. If necessary: Neutralize the residue with a dilute solution of acetic acid. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.

### Large Spill:

Corrosive solid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Neutralize the residue with a dilute solution of acetic acid. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

## Section 7: Handling and Storage

### Precautions:

Keep container dry. Do not ingest. Do not breathe dust. Never add water to this product. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as moisture.

**Storage:** Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 23°C (73.4°F).

## Section 8: Exposure Controls/Personal Protection

### Engineering Controls:

Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

**Personal Protection:** Safety glasses. Synthetic apron. Gloves (impervious).

### Personal Protection in Case of a Large Spill:

Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

### Exposure Limits:

TWA: 15 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Inhalation Total. TWA: 5 (mg/m<sup>3</sup>) from OSHA (PEL) [United States] Inhalation Respirable. TWA: 5 STEL: 5 (mg/m<sup>3</sup>) from AIHA Inhalation Consult local authorities for acceptable exposure limits.

## Section 9: Physical and Chemical Properties

**Physical state and appearance:** Solid.

**Odor:** Odorless.

**Taste:** Not available.

**Molecular Weight:** 163.94 g/mole

**Color:** White.

**pH (1% soln/water):** 11.9 [Basic.]

**Boiling Point:** Not available.

**Melting Point:** 75°C (167°F)

**Critical Temperature:** Not available.

**Specific Gravity:** 1.62 (Water = 1)

**Vapor Pressure:** Not applicable.

**Vapor Density:** Not available.

**Volatility:** Not available.

**Odor Threshold:** Not available.

**Water/Oil Dist. Coeff.:** Not available.

**Ionicity (in Water):** Not available.

**Dispersion Properties:** See solubility in water.

### Solubility:

Easily soluble in hot water. Soluble in cold water.

## Section 10: Stability and Reactivity Data

**Stability:** The product is stable.

**Instability Temperature:** Not available.

**Conditions of Instability:** Moisture

**Incompatibility with various substances:** Reactive with moisture.

**Corrosivity:** Non-corrosive in presence of glass.

**Special Remarks on Reactivity:** Hygroscopic. Sodium Phosphate Tribasic forms a strong caustic solution similar to soda lye

**Special Remarks on Corrosivity:** When wet, mild steel and brass may be corroded by sodium phosphate tribasic.

**Polymerization:** Will not occur.

## Section 11: Toxicological Information

**Routes of Entry:** Absorbed through skin. Inhalation. Ingestion.

### Toxicity to Animals:

Acute oral toxicity (LD50): 4150 mg/kg [Rat [information from other supplier]]. Acute dermal toxicity (LD50): >300 mg/kg [Rabbit [Registry of Toxic Effects of Chemical Substances database]].

**Chronic Effects on Humans:** Not available.

### Other Toxic Effects on Humans:

Extremely hazardous in case of skin contact (corrosive), of eye contact (corrosive), of inhalation (lung corrosive). Hazardous in case of skin contact (irritant), of ingestion, . Slightly hazardous in case of skin contact (permeator).

**Special Remarks on Toxicity to Animals:** Not available.

**Special Remarks on Chronic Effects on Humans:** May affect genetic material (mutagenic)

### Special Remarks on other Toxic Effects on Humans:

Acute Potential Health Effects: Skin: Causes skin irritation with possible burning pain and corrosive damage. It may be absorbed through the skin. Eyes: Causes eye irritation. It causes immediate and severe pain followed by conjunctival edema and corneal clouding. Later cataract formation may occur. This substance may cause eye burns. Inhalation: May be harmful if inhaled. Inhalation of dust may Cause respiratory tract and mucous membrane irritation with coughing, sneezing, choking, difficulty breathing, and pulmonary edema. Ingestion: May be harmful if swallowed. May cause severe gastrointestinal (digestive) tract irritation with severe nausea, vomiting, abdominal discomfort, violent purging, diarrhea, and burning sensation. Ingestion of large amounts may induce hypocalcemia or hyponatremia characterized by tetanus-like spasms, due to the sequestration of calcium ions by the phosphate moiety. It may also cause caustic burns of the mouth oropharynx, esophagus, or gastrointestinal tract.

## Section 12: Ecological Information

### Ecotoxicity:

Ecotoxicity in water (LC50): 220 mg/l 96 hours [Bluegill sunfish]. 120 mg/l 96 hours [Rainbow Trout]. 177 mg/l 50 hours [Daphnia].

**BOD5 and COD:** Not available.

### Products of Biodegradation:

Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

**Toxicity of the Products of Biodegradation:** The products of degradation are as toxic as the original product.

**Special Remarks on the Products of Biodegradation:** Not available.

## Section 13: Disposal Considerations

**Waste Disposal:**

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

**Section 14: Transport Information**

**DOT Classification:** Not a DOT controlled material (United States).

**Identification:** : Not available. UNNA: 9148 PG: III

**Special Provisions for Transport:** Not applicable.

**Section 15: Other Regulatory Information****Federal and State Regulations:**

New York release reporting list: Sodium phosphate tribasic Pennsylvania RTK: Sodium phosphate tribasic Minnesota: Sodium phosphate tribasic Massachusetts RTK: Sodium phosphate tribasic New Jersey: Sodium phosphate tribasic California Director's List of Hazardous Substances: Sodium phosphate tribasic TSCA 8(b) inventory: Sodium phosphate tribasic CERCLA: Hazardous substances.: Sodium phosphate tribasic: 5000 lbs. (2268 kg)

**Other Regulations:**

OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200). EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

**Other Classifications:**

**WHMIS (Canada):** CLASS E: Corrosive solid.

**DSCL (EEC):**

R35- Causes severe burns. S1/2- Keep locked up and out of the reach of children. S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36/37- Wear suitable protective clothing and gloves. S39- Wear eye/face protection. S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

**HMIS (U.S.A.):**

**Health Hazard:** 2

**Fire Hazard:** 0

**Reactivity:** 0

**Personal Protection:** C

**National Fire Protection Association (U.S.A.):**

**Health:** 2

**Flammability:** 0

**Reactivity:** 2

**Specific hazard:**

**Protective Equipment:**

Gloves (impervious). Synthetic apron. Wear appropriate respirator when ventilation is inadequate. Safety glasses.

**Section 16: Other Information**

**References:** Not available.

**Other Special Considerations:** Not available.

**Created:** 10/09/2005 06:35 PM

**Last Updated:** 05/21/2013 12:00 PM

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# Material Safety Data Sheet

## TEAc-N Perfluoroalkyl Ethyl Acrylates (Narrow Distribution)

Revised 03- November-2009

### 1. PRODUCT AND COMPANY IDENTIFICATION

#### Material Identification

**Product name:** TEAc-N (narrow distribution)  
**Chemical name:** Perfluoroalkyl ethyl acrylates, mixture  
*1H,1H,2H,2H*-Perfluoroalkyl-1-acrylates  
**Chemical formula:**  $\text{CH}_2=\text{CHC}(\text{O})\text{OCH}_2\text{CH}_2(\text{CF}_2\text{CF}_2)_n\text{F}$ ,  $n = 3, 4, 5$  ;  
mostly  $n = 4$   
 $\text{C}_n\text{H}_7\text{F}_m\text{O}_2$ ;  $n = 9, 11, 13, 15$  ;  $m = 2n-9$

#### Company Identification

**Distributor** Top Fluorochem Co., Ltd.  
Building C2, 3<sup>rd</sup> Floor  
479 Chun Dong Road  
XinZhuang Industrial Park  
Minhang District  
Shanghai, P. R. China 201108

**Manufacturer** Fuxin Hengtong Fluorine Chemicals Co., Ltd.  
Fuxin Chemicals Community  
West Pingan St.  
Haizhou District  
Fuxin City, Liaoning Province, P. R. China

**Emergency Call** +86-21-54833399

### 2. COMPOSITION AND INFORMATION ON INGREDIENTS

#### Components

Material	CAS Number	EINECS	TSCA Listed	Amount
Perfluoroalkylethyl acrylates	65605-70-1	NA	NA	
2,6-Di- <i>tert</i> -butyl-4-methylphenol	128-37-0	204-881-4	Yes	30 ppm

### 3. HAZARDS IDENTIFICATION

#### Potential Health Effects

**Skin contact:** May cause irritation.  
**Eye contact:** May cause irritation.  
**Inhalation of spray or mist:** May cause nasal, throat, or lung irritation.  
**Inhalation of large amounts:** May be toxic to the lungs.  
**Symptoms:** May be modest initially, followed in hours by severe

shortness of breath requiring prompt medical attention.

**Carcinogenicity Information:**

None of the components present in this material at concentrations equal to or greater than 0.1 % are listed by IARC, NTP, OSHA or ACGIH as a carcinogen.

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**4. FIRST AID MEASURES**

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<b>Inhalation:</b>	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.
<b>Skin Contact:</b>	Flush skin with water after contact. Wash contaminated clothing before reuse.
<b>Eye Contact:</b>	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.
<b>Ingestion:</b>	If swallowed, do not induce vomiting. Immediately give 2 glasses of water. Never give anything by mouth to an unconscious person. Call a physician.
<b>Note to Physicians:</b>	Activated charcoal mixture may be administered. To prepare activated charcoal mixture, suspend 50 grams activated charcoal in 400 mL water and mix thoroughly. Administer 5 mL/kg, or 350 mL for an average adult.

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**5. FIRE FIGHTING MEASURES**

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**Flammable Properties**

<b>Flash point:</b>	> 110 °C (> 230 °F)
<b>Method:</b>	PMCC

<b>Hazardous Decomposition Products:</b>	Hazardous decomposition products include carbon dioxide, carbon monoxide, and hydrogen fluoride. Toxic gases or particles may be formed during combustion. These products may cause severe eye, nose, throat, and lung irritation or toxic effects.
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<b>Extinguishing Media:</b>	Water spray, foam, dry chemical, CO <sub>2</sub> .
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<b>Fire Fighting Instructions:</b>	Evacuate personnel to a safe area. Keep personnel removed and upwind of fire. Wear self-contained breathing apparatus. Wear full protective equipment. Avoid breathing decomposition products.
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**6. ACCIDENTAL RELEASE MEASURES**

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**Safeguards (Personnel)**

<b>NOTE:</b>	Review FIRE FIGHTING MEASURES and HANDLING (Personnel) sections before proceeding with clean-up. Use appropriate personal protective equipment during clean-up.
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<b>Spill clean up:</b>	Soak up with sawdust, sand, oil dry or other absorbent material. Shovel or sweep up.
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**7. HANDLING AND STORAGE**


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**Handling Requirements:**

Avoid breathing vapors or mist.

Avoid contact with eyes, skin, or clothing.

Wash thoroughly after handling.

Do not store or consume food, drink, or tobacco in areas where they may become contaminated with this material.

Avoid circumstances that produce respirable particles unless suitable ventilation and respirator are used.

**Storage conditions:**Keep container and store in a cool place.  
Keep container tightly closed.

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**8. EXPOSURE CONTROLS AND PERSONAL PROTECTION**


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**Engineering Controls:**

Keep container tightly closed. Use only with adequate ventilation. Vent heated extruder or dryer fumes outside work area. Do not aerosolize. In spray applications, use airless type pressure spray equipment at less than 60 psi, and exhaust ducts, drip pans, or other design features to minimize worker exposure to mists and overspray.

**Personal Protective Equipment****Eye/face protection:**

Wear safety glasses or cover-all chemical splash goggles.

**Respirators:**

Where there is potential for airborne exposures wear NIOSH approved respiratory protection.

**Protective clothing:**

Where there is potential for skin contact have available and wear as appropriate impervious gloves, apron, pants, and jacket.

**Exposure Guidelines****PEL (OSHA):**

None established.

**TLV (ACGIH):**

None established.

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**9. PHYSICAL AND CHEMICAL PROPERTIES**


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**Boiling Point**100 to 220 °C (212 to 428 °F) @ 10 mm Hg  
Approx.**Melting Point**

-1 to 25 °C

**Flash Point**

&gt; 110 °C

**pH**

3 to 5 @ 20 °C

**Density**

1.6 g/mL @ 25 °C

**Odor**

Mild acrylic

**Form**

Semi-solid

**Color**

Pale to medium yellow

**Refractive Index** $n_D^{25} = 1.3332$ **Solubility in Water**

Negligible



**10. STABILITY AND REACTIVITY**

<b>Chemical Stability:</b>	Stable at normal temperatures and storage conditions. Air inhibits polymerization.  Incompatible with peroxides and free radical sources.
<b>Decomposition:</b>	Decomposes with heat. Hazardous decomposition products include carbon dioxide, carbon monoxide, and hydrogen fluoride. Toxic gases or particles may be formed during combustion. These products may cause severe eye, nose, throat, and lung irritation or toxic effects.
<b>Polymerization:</b>	Polymerization can occur. Conditions leading to polymerization are free radical sources, heat, and UV light.

**11. ECOLOGICAL INFORMATION**

<b>General:</b>	Take care to prevent chemicals from entering the ground, water courses or drainage systems.
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**12. DISPOSAL CONSIDERATIONS**

<b>Disposal Operations:</b>	Material should be disposed of in accordance with local, state and federal regulations.
<b>Disposal of Packaging:</b>	Dispose of as special waste in compliance with local and national regulations. Observe all federal, state and local environmental regulations.  The user's attention is drawn to the possible existence of regional or national regulations regarding disposal.

**13. TRANSPORTATION INFORMATION**

<b>Mode</b>	DOT/IMDG/IATA
<b>UN Number</b>	None. Non-hazardous for transport.
<b>Class (Subsidiary)</b>	None. Non-hazardous for transport.
<b>Proper Shipping Name</b>	None. Non-hazardous for transport.
<b>Hazard Label (Subsidiary)</b>	None. Non-hazardous for transport.
<b>Packing Group</b>	None. Non-hazardous for transport.
<b>Shipping Hazard Label</b>	None. Non-hazardous for transport.

**14. REGULATORY INFORMATION**

<b>U.S. Federal Regulations</b>	TITLE III  HAZARD CLASSIFICATIONS SECTIONS 311, 312
<b>Acute :</b>	Yes
<b>Chronic :</b>	No
<b>Fire :</b>	No

**Reactivity :** No

**Pressure :** No

**Incompliance with TSCA Inventory requirements.**

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## 15. OTHER INFORMATION

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**Personal Protection rating:** To be supplied by user depending on use conditions.

**NPCA-HMIS Rating:**

Health: 2

Flammability: 1

Reactivity: 1

**Legal Disclaimer:**

For R&D use only. Not for drug, household, or other uses. The previous information is based upon our current knowledge and experience of our product and is not exhaustive. It applies to the product as defined by the specifications. In case of combinations or mixtures, one must confirm that no new hazards are likely to exist. In any case, the user is not exempt from observing all legal, administrative and regulatory procedures relating to the product, personal hygiene, and integrity of the work environment. Unless noted to the contrary, the technical information applies only to pure product.

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End Of MSDS

Revised February 23, 2004  
Supersedes March 22, 2002 revision

## MATERIAL SAFETY DATA SHEET

### 1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

**PRODUCT NAME:** TMAC

**SYNONYMS:** N,N,N – Trimethyl Methanaminium Chloride  
USAF AN-8  
Tetramethylammonium Chloride

**MANUFACTURER:** BCP Ingredients, Inc.  
299 Extension Street  
Verona, MO 65769-0085  
(417) 498-2241 [USA]

**EMERGENCY CONTACT:** (417) 498-2241 [USA] – Facility  
(800) 424-9300 [USA] – Chemtrec

### 2. COMPOSITION/INFORMATION ON INGREDIENTS

<u>COMPONENTS</u>	<u>WEIGHT %</u>	<u>CAS #</u>	<u>EXPOSURE LIMITS</u>	<u>CITATION</u>
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H <sub>2</sub> O	40 - 69			
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#### HAZARDOUS

<u>COMPONENTS</u>	<u>WEIGHT %</u>	<u>CAS #</u>	<u>EXPOSURE LIMITS</u>	<u>CITATION</u>
-------------------	-----------------	--------------	------------------------	-----------------

(CH <sub>3</sub> ) <sub>4</sub> NCl	31 - 60	75-57-0	Not Established	
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### 3. HAZARDS IDENTIFICATION

#### Emergency Overview

Clear liquid; odorless to slight amine (fish-like) odor. Irritating to eyes, respiratory tract and skin. When heated to decomposition, releases very toxic fumes of nitrogen oxides (NO<sub>x</sub>) and chlorides (Cl). Poison by intraperitoneal, subcutaneous and other unspecified routes.

#### Potential Health Effects

**Eye:** May cause eye irritation or chemical burn.

**Inhalation:** Breathing vapors may cause respiratory irritation.

**Skin:** May cause skin irritation.

**Ingestion:** May be fatal if swallowed. It may cause rapid removal of calcium from the body. May chelate lead in the gastrointestinal tract causing absorption.

**Systemic:** No known physiological hazards.

**Medical Conditions Aggravated by Exposure:** None determined.

**Exposure Symptoms:**

Acute –	Eye: Burning, itching or pain Inhalation: Nose and throat irritation, coughing, dizziness, headache, drowsiness. Skin: Mild (redness) to severe (blisters) irritation depending on extent of contact. Ingestion: Irritation of the mouth, throat and stomach including nausea, vomiting, diarrhea, and abdominal pain. May cause numbness and tingling sensations; urinary frequency, chills, fever and arthralgia
Chronic –	Eye: May cause conjunctivitis Inhalation: None determined Skin: May cause dermatitis Ingestion: High doses of pure salt have induced severe renal tubular lesions in man and animal

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## 4. FIRST AID MEASURES

**Eye:** Flush immediately with clean, low-pressure water for at least 15 minutes while occasionally lifting eyelids. Notify supervisor and seek medical attention.

**Inhalation:** No emergency care anticipated. If there is difficulty breathing, remove to fresh air and get medical attention.

**Skin:** Wash immediately with soap and water. Remove contaminated clothing and wash contacted skin with soap and water. If irritation occurs or persists, check with medical personnel. Wash contaminated clothing before reuse.

**Ingestion:** Seek medical attention immediately. If victim is conscious, give up to two quarts of water and induce vomiting. Do not give anything by mouth if the victim is drowsy, unconscious, or has no gag reflex.

**Note to Physician:** If medical attention is sought, treatment should be based on the judgement of the physician in response to the reactions of the patient.

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## 5. FIRE FIGHTING MEASURES

**Flammable Properties:** Flash point – >200°F.; Method – closed cup

**Flammable Limits:** Lower Flammable Limit (LFL) – not determined  
Upper Flammable Limit (UFL) – not determined

**Auto Ignition Temperature:** Not determined

**Hazardous Combustion Products:** No specific hazards. Combustion will produce compounds of carbon, hydrogen, nitrogen and oxygen. Hydrogen chloride could also potentially be produced. The exact composition of the products of combustion will depend on the conditions of combustion.

**Other Fire and Explosion Hazards:** None.

**Extinguishing Media:** Water, Foam, CO<sub>2</sub>, Dry Chemical.



**Fire Fighting Equipment:** Full protective equipment (Bunker Gear) and NIOSH/MSHA approved SCBA should be used for all fires.

**Fire Fighting Instructions:** This material may burn, but it does not readily ignite. Use water spray to cool sealed drums surrounded by a fire to prevent bursting from steam pressure. Material is incompatible with strong oxidizers. Water run off can cause environmental damage. Dike and collect water used to fight fires.

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## 6. ACCIDENTAL RELEASE MEASURES

Use absorbent materials to contain and collect spilled material. Place used absorbent in a disposal container.

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## 7. HANDLING AND STORAGE

### General Handling Precautions

Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Avoid breathing vapors. Ensure containers are properly secured before moving.

### Storage Information

Storage temperature: Ambient recommended. Do not allow to freeze.

**Shelf life:** No known limit. Use within 1 year recommended.

**Special Sensitivity:** None

**Miscellaneous:** Material is incompatible with strong oxidizers.

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## 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

**Engineering Controls:** None. Local and general exhaust recommended.

**Eye Protection:** Use safety glasses with side shields, chemical safety goggles and/or face shield. The choice of protection should be appropriate to the task being performed and risk of splashing.

**Respiratory Protection:** For most conditions, no respiratory protection should be needed. An organic vapor/acid gas cartridge with a dust/mist filter may be used if desired. In confined or poorly ventilated areas or emergency conditions, use an approved positive pressure self-contained breathing apparatus.

**Skin Protection:** Adequate skin protection should be provided at all times to minimize skin contact. Use rubber gloves, rubber aprons, splash suits, and/or sleeve protectors. The choice of protection should be appropriate to the task being performed and risk of splashing.

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## 9. PHYSICAL AND CHEMICAL PROPERTIES

<b>Chemical Family:</b>	Quaternary salt
<b>Appearance:</b>	Clear
<b>Physical state:</b>	Liquid
<b>Odor:</b>	Odorless to slight amine odor

<b>Molecular Formula:</b>	C <sub>4</sub> H <sub>12</sub> NCI
<b>Molecular Weight:</b>	109.62
<b>Specific Gravity:</b>	1.0153
<b>Bulk Density:</b>	8.50 pounds/gallon
<b>Solubility:</b>	Soluble
<b>Octanol/Water Partition Coefficient:</b>	
	Not determined (assumed to be 0)
<b>pH:</b>	7 (typical)
<b>Melting Point:</b>	Not determined
<b>Boiling Point:</b>	216°F. (102°C.) at 760 mmHg
<b>Decomposition Point:</b>	788°F (420°C) for pure salt
<b>Evaporation Rate:</b>	Not determined.
<b>VOC Content:</b>	45 – 50% volatile by volume including water.
<b>Vapor Pressure:</b>	25 mmHg
<b>Vapor Density:</b>	<1
<b>Viscosity:</b>	6 cps

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## 10.STABILITY AND REACTIVITY

<b>Chemical Stability:</b>	Stable under normal conditions.
<b>Material Incompatibility:</b>	Incompatible with strong oxidizers.
<b>Hazardous Decomposition Products:</b>	Compounds of carbon, hydrogen, nitrogen and oxygen. Potentially could produce hydrogen chloride gas.
<b>Hazardous Polymerization:</b>	None

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## 11.TOXICOLOGICALY INFORMATION

Mysidopsis bahia: 96-hour, 10.5 ppm  
LD<sub>50</sub> = 220 mg/kg (for male rats)

### Pure salt

LD<sub>LO</sub> = 125mg/kg oral (mouse)  
LD<sub>50</sub> = 25mg/kg intraperitoneal (mouse)  
LD<sub>50</sub> = 40mg/kg subcutaneous (mouse)  
LD<sub>LO</sub> = 20mg/kg (mouse)  
LD<sub>LO</sub> = 6mg/kg subcutaneous (rabbit)  
LD<sub>LO</sub> = 20mg/kg (guinea pig)  
LD<sub>LO</sub> = 2gm/kg subcutaneous (frog)

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## 12.ECOLOGICAL INFORMATION

Biodegrades slowly.

LC<sub>50</sub> = 462,000 mg/L pure salt for fathead minnow (*Pimephales promelas*)

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## 13.DISPOSAL CONSIDERATIONS

Not considered a hazardous waste under Federal Hazardous Waste Regulations (40 CFR 261). Liquid solutions should be solidified or absorbed, and should be landfilled after securing Environmental Regulatory Agency and landfill operations approval. Containers should be emptied completely and have all closures in place. Return containers for reuse, or dispose in a landfill after securing Environmental Regulatory Agency and landfill operations approval. Consult state and local regulations regarding proper disposal as they may be more restrictive or otherwise different from Federal regulations.

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## 14.TRANSPORT INFORMATION

D.O.T. Hazardous Material (49 CFR 172.101).

**Shipping Description:** Toxic liquid, organic, n.o.s., 6.1, UN2810, PGIII (contains tetramethylammonium chloride).

**Labeling:** Poison

**Emergency Response Guide Number:** 153

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## 15.REGULATORY INFORMATION

### U.S. Federal Regulations

**OSHA:** This product is hazardous under the criteria of the Federal OSHA Hazard Communication Standard 29 CFR 1910.1200.

**PSM:** This product is not subject to Process Safety Management (29 CFR 1910.119).

**FIFRA:** Not applicable.

**TSCA:** On TSCA inventory.

**CERCLA:** Reportable Quantity – None (40 CFR 302.4).

**SARA TITLE III:** Section 302 Extremely Hazardous Substances – None (40 CFR 355).  
Section 311/312 Hazard Categories – Immediate chronic mixture (40 CFR 370.2)  
Section 313 Toxic Chemicals – None (40 CFR 372.65)

**RMP:** Not listed under the Risk Management Plan (40 CFR 68).

**RCRA:** If discarded in purchased form, this product is not a listed or characteristic hazardous waste. However, under RCRA, it is the responsibility of the product user to determine at the time of disposal whether a material containing the product or derived from the product should be classified as a hazardous waste (40 CFR 261.20-24).

**CWA:** Release into a waterway may require reporting to the National Response Center @ 800-424-8802 (40 CFR 116.4).

**FDA/USDA:** Follow Good Manufacturing Practice (GMP).



### International Regulations

**Canadian Dangerous Substance List (DSL):** Not available.

**European Inventory of Existing Commercial Chemical Substances (EINECS):** Not available.

**Australian Inventory of Chemical Substances (AICS):** Not available.

**Korean Existing Chemicals List (ECL):** Not available.

### State Regulations

California Proposition 65: Not listed.

There are no known additional requirements necessary for compliance with state right-to-know regulations.

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## 16. OTHER INFORMATION

**Reason for Issue:** Revision to Section 1, Manufacturer's name changed.

**Hazard Ratings** - The following hazard ratings are recommended for this product:

NFPA	
Fire	- 1
Health	- 3
Reactivity	- 0
Specific Hazard	- None

(NFPA Scale: 4 = severe, 3 = serious, 2 = moderate, 1 = slight, 0 = minimal)

**Abbreviations** - The following abbreviations are used in this document:

% - percent

cps - centipoises

gm/kg - grams per kilogram

mg/kg - milligrams per kilogram

mmHg - milligrams per cubic meter

mg/m<sup>3</sup> - milligrams per cubic meter

n.o.s. - not otherwise specified

ppm - parts per million

µg/L - micrograms per liter

ACGIH - American Council of Governmental Industrial Hygienists

AICS - Australian Inventory of Chemical Substances

CAS - Chemical Abstract Service

CERCLA - Comprehensive Emergency Response, Compensation and Liability Act

CFR - Code of Federal Regulations

CWA - Clean Water Act

D.O.T. - Department of Transportation

DSL - Domestic Substance List (Canada)

ECL - Existing Chemicals List (Korea)

EINECS - European Inventory of Existing Commercial Substances

FDA - Food and Drug Administration

FIFRA - Federal Insecticide, Fungicide and Rodenticide Act

IDLH - Immediately Dangerous to Life and Health

LC<sub>50</sub> - Lethal concentration fifty; a calculated concentration of a substance in air, exposure to which for a specified length of time is expected to cause death of 50% of a laboratory animal population.

LD<sub>LO</sub> - Lethal dose low; the lowest dose of a substance introduced by any route other than inhalation reported to have caused death in humans or animals.



LD<sub>50</sub> – Lethal dose fifty; the dose of a substance expected to cause 50% mortality of an experimental animal population.

LFL - Lower Flammable Limit

MSHA - Mine Safety Health Administration

NFPA - National Fire Protection Association

NIOSH - National Institute of Occupational Safety and Health

OSHA - Occupational Safety and Health Administration

PEL - Permissible Exposure Limit (default 8-hour day, 40-hour week TWA)

PSM - Process Safety Management

RCRA - Resource Conservation and Recovery Act

REL - Recommended Exposure Limit (default 10-hour day, 40-hour week TWA)

RMP - Risk Management Plan

SARA - Superfund Amendment and Reauthorization Act

STEL - Short Term Exposure Limit (default 15-minute TWA)

TSCA - Toxic Substance Control Act

TWA - Time Weighted Average

UFL - Upper Flammable Limit

USDA - United States Department of Agriculture

\*\*\*\*\*  
This information is furnished without warranty, expressed or implied, regarding this information, the results to be obtained from the use thereof, or the hazards connected with the use of this material, except that it is accurate to the best knowledge of BCP Ingredients, Inc.. The data on this MSDS relate only to the specific material designated herein. Final determination of the suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, since the product may be subjected to conditions beyond our control and with which we may be unfamiliar, we cannot guarantee that these are the only hazards which exist. Nor can we assume any responsibility for the results of the use of this data. It is expected that the persons receiving this data shall make their own determination of the effects, properties, and protections which pertain to their particular situation.  
\*\*\*\*\*

Prepared by: EH&S Department (417) 498-2241 [USA]

# Toxic liquids, organic, n.o.s. (contains tetramethylammonium chloride)

**GUIDE**  
**153**

**SUBSTANCES - TOXIC AND/OR CORROSIVE**  
**(COMBUSTIBLE)**

**ERG2008**

**ERG2008**

**SUBSTANCES - TOXIC AND/OR CORROSIVE**  
**(COMBUSTIBLE)**

**GUIDE**  
**153**

## POTENTIAL HAZARDS

### HEALTH

- **TOXIC:** Inhalation, ingestion or skin contact with material may cause severe injury or death.
- Contact with molten substance may cause severe burns to skin and eyes.
- Avoid any skin contact.
- Effects of contact or inhalation may be delayed.
- Fire may produce irritating, corrosive and/or toxic gases.
- Runoff from fire control or dilution water may be corrosive and/or toxic and cause pollution.

### FIRE OR EXPLOSION

- Combustible material: may burn but does not ignite readily.
- When heated, vapors may form explosive mixtures with air: indoors, outdoors and sewers explosion hazards.
- Those substances designated with a "P" may polymerize explosively when heated or involved in a fire.
- Contact with metals may evolve flammable hydrogen gas.
- Containers may explode when heated.
- Runoff may pollute waterways.
- Substance may be transported in a molten form.

## PUBLIC SAFETY

- **CALL Emergency Response Telephone Number on Shipping Paper first. If Shipping Paper not available or no answer, refer to appropriate telephone number listed on the inside back cover.**
- As an immediate precautionary measure, isolate spill or leak area in all directions for at least 50 meters (150 feet) for liquids and at least 25 meters (75 feet) for solids.
- Keep unauthorized personnel away.
- Stay upwind. • Keep out of low areas. • Ventilate enclosed areas.

### PROTECTIVE CLOTHING

- Wear positive pressure self-contained breathing apparatus (SCBA).
- Wear chemical protective clothing that is specifically recommended by the manufacturer. It may provide little or no thermal protection.
- Structural firefighters' protective clothing provides limited protection in fire situations ONLY; it is not effective in spill situations where direct contact with the substance is possible.

### EVACUATION

#### Spill

- See Table 1 - Initial Isolation and Protective Action Distances for highlighted materials. For non-highlighted materials, increase, in the downwind direction, as necessary, the isolation distance shown under "PUBLIC SAFETY".

#### Fire

- If tank, rail car or tank truck is involved in a fire, ISOLATE for 800 meters (1/2 mile) in all directions; also, consider initial evacuation for 800 meters (1/2 mile) in all directions.

## EMERGENCY RESPONSE

### FIRE

- **Small Fire**
  - Dry chemical, CO<sub>2</sub> or water spray.
- **Large Fire**
  - Dry chemical, CO<sub>2</sub>, alcohol-resistant foam or water spray.
  - Move containers from fire area if you can do it without risk.
  - Dike fire-control water for later disposal; do not scatter the material.
- **Fire Involving Tanks or Car/Trailer Loads**
  - Fight fire from maximum distance or use unmanned hose holders or monitor nozzles.
  - Do not get water inside containers.
  - Cool containers with flooding quantities of water until well after fire is out.
  - Withdraw immediately in case of rising sound from venting safety devices or discoloration of tank.
  - ALWAYS stay away from tanks engulfed in fire.

### SPILL OR LEAK

- **ELIMINATE** all ignition sources (no smoking, flares, sparks or flames in immediate area).
- Do not touch damaged containers or spilled material unless wearing appropriate protective clothing.
- Stop leak if you can do it without risk.
- Prevent entry into waterways, sewers, basements or confined areas.
- Absorb or cover with dry earth, sand or other non-combustible material and transfer to containers.
- **DO NOT GET WATER INSIDE CONTAINERS.**

### FIRST AID

- Move victim to fresh air. • Call 911 or emergency medical service.
- Give artificial respiration if victim is not breathing.
- **Do not use mouth-to-mouth method if victim ingested or inhaled the substance; give artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device.**
- Administer oxygen if breathing is difficult.
- Remove and isolate contaminated clothing and shoes.
- In case of contact with substance, immediately flush skin or eyes with running water for at least 20 minutes.
- For minor skin contact, avoid spreading material on unaffected skin.
- Keep victim warm and quiet.
- Effects of exposure (inhalation, ingestion or skin contact) to substance may be delayed.
- Ensure that medical personnel are aware of the material(s) involved and take precautions to protect themselves.





## Material Safety Data Sheet for Hamster Cell Cultures (Biosafety Level 1)

### 1. Product Identification

Name of cell line: CHO-K1 (CLS order no. 603480)  
Designation: Chinese-Hamster-Ovary cell line, permanent cell line

### 2. Company Identification

CLS Cell Lines Service GmbH  
Dr. Eckener-Str. 8  
D-69214 Eppelheim  
Germany

Emergency phone number: +49 (0)6221 700799

### 3. Composition / Ingredients

Unit: cryovial; frozen liquid

Hazardous Ingredient(s)	CAS no.	Percentage	EC no.
Dimethyl Sulfoxide	67-68-5	10	200-664-3
Non-Hazardous Ingredient(s)		Percentage	
DMEM, supplemented for freezing		60-80	
FBS (Fetal Bovine Serum)		10-20	
Cells		1	

### 4. Hazards Identification

Chinese-Hamster source cell line  
Categorized as non-infectious and non-toxic

### 5. First Aid Measures

Skin contact:  
Wash off immediately with plenty of water and soap.  
Eye contact:  
Flush eyes immediately with water for 10-15 minutes.  
Ingestion:  
If the material was swallowed, rinse the mouth with water.

### 6. Accidental Release Measures

Use personal protective equipment  
Do not flush into surface water.  
Clean contaminated surface thoroughly. Autoclave before disposal into appropriated containers.

## 7. Handling and storage

### Handling:

Open only under a sterile workbench. Wear protective equipment. Handle as if containing infectious material.

### Storage:

Keep the cryovial at -150°C (freezer) or at -196°C (liquid nitrogen vapour phase).

## 8. Personal Protection

### Hygienic measures

Avoid the contact with skin, eyes and clothing. Keep away from food and drinks. Wash hands immediately after handling the product.

Normally, no respiratory protective equipment is required.

Use protective gloves and safety goggles and wear a lab coat while handling the product.

## 9. Physical and Chemical Properties / Reactivity

### Form:

Liquid

DMSO is stable. It is incompatible with a very wide range of materials, including acid chlorides, strong acids, strong oxidizing agents, strong reducing agents, phosphorus halides, moisture, copper wool + trichloroacetic acid, hygroscopic.

## 10. Toxicological Information

Not hazardous according to Directive 67/548/EC.

### Toxicity data for DMSO:

ORL-RAT LD50 14500 mg kg<sup>-1</sup>  
IVN-MAN TDLO 686 mg kg<sup>-1</sup>  
IVN-MUS LD50 3100 mg kg<sup>-1</sup>  
IVN-DOG LD50 2500 mg kg

ORL-MAM LD50 21400 mg kg<sup>-1</sup>  
IPR-RAT LD50 8200 mg kg<sup>-1</sup>  
ORL-BWD LD50 100 mg kg<sup>-1</sup>

## 11. Transportation Information

Non-hazardous for air, sea and road freight.

## 12. General Information

### Recommended use:

For in vitro research use only.

### Disclaimer:

The information provided in the present Material Safety Data Sheet is believed to be correct at the date of publication. No guarantee is given for its accuracy or completeness, but is intended as guidance only.

Biological material may be hazardous and should be used with caution.

CLS · Cell Lines Service shall not be held liable for any damage resulting from handling or from contact with the product.